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Title of Thesis: "Behavioral effects of enrichment and nicotine in male Sprague Dawley rats"

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ABSTRACT

Title of Thesis: "Behavioral effects of enrichment and nicotine in male Sprague Dawley rats"

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Three experiments examined effects of environmental enrichment and nicotine on body weight, food consumption, and activity in 64 male Sprague Dawley rats. Rats were housed in enriched (physical, social, or super [social and physical enrichment]) or non-enriched environments. Half of the animals received nicotine for 18 days. Rats in the super-enriched group, compared with rats in the other housing groups, had: attenuated body weight gain, decreased home cage activity, decreased open-field locomotor activity, increased habituation to a novel environment, decreased voluntary exercise. Rats in the physically-enriched group had increased voluntary exercise compared with the other housing groups. Rats in the nicotine group, compared with the saline group, had decreased body weight and increased voluntary exercise. Environmental enrichment prolonged nicotine's effects through nicotine cessation. Enrichment's effects on body weight could not be explained by food

consumption and activity. These findings and their implication for behavioral health are discussed.

Behavioral Effects of Enrichment and Nicotine in Male Sprague Dawley Rats

by

Stephanie M. Long

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Overview

Tobacco use, body weight, physical activity, and environmental influences all are recognized to be important to health. Each of these topics has received extensive research attention (U.S. Department of Health and Human Services, 1996c; U.S. Department of Health and Human Services, 2000d; U.S. Department of Health and Human Services, 2001b; U.S. Department of Health and Human Services, 2006a; U.S. Department of Health and Human Services, 2006b). Some of these topics have been studied together to understand how they may affect each other or how they may act together to affect health (Grunberg, 1992). For example, tobacco use, physical activity, and environmental influences each affect body weight. The present series of experiments was designed to examine the effects of all of these health-relevant variables together. Specifically, three laboratory experiments were conducted to examine: (1) environmental influences (especially social and physical enrichment) on activity and body weight; (2) environmental influences and nicotine (the drug of addiction in tobacco) administration on activity and body weight; and (3) environmental influences and nicotine cessation on activity and body weight. An animal (rat) model was used to carefully control variables under study and because manipulation of nicotine to drug-naïve subjects would not be ethical in humans.

As background for the present research, this master's thesis briefly reviews relevant information about tobacco use, obesity, physical activity, and environmental enrichment. Then, each of the three experiments is presented including hypotheses, methods, results, and discussion. Next, a general

discussion pulls the findings together and addresses limitations and future directions.

Tobacco Use

Tobacco use is the leading preventable cause of death in the United States and around the world, accounting for roughly 440,000 deaths/year in the U.S. and 5 million/year worldwide (Centers for Disease Control and Prevention, 2007a; Centers for Disease Control and Prevention, 2007b). Cigarette smoking leads to a variety of chronic health conditions, including cardiovascular diseases, cancers, chronic obstructive pulmonary diseases, and immunosuppression. Despite the dramatic death toll and well-publicized health hazards of tobacco use, approximately 20% of American adults smoke and approximately 20% worldwide.

Tobacco use is maintained by biological, psychological, social, and environmental factors. Nicotine is a highly addictive psychoactive alkaloid found in tobacco (Grunberg, 1986). Nicotine self-administration in tobacco products has direct, biological actions that are rewarding via dopaminergic pathways in the ventral tegmental (VTA) area of the brain similar to effects of other drugs, including cocaine and amphetamines (Koob & Le Moal, 2006; Tapper et al., 2006). In addition, nicotine affects body weight, food consumption, metabolism, attention, information processing, and pain in ways that many people find to be desirable (Ditre & Brandon, 2008; Grunberg, 1985; Grunberg, 1997; Piper et al., 2008). Peer influences, associations made through advertising, and other social and psychological variables also reinforce tobacco use in humans (Jarvis, 2004).

Biological and behavioral variables relevant to tobacco use and the role of nicotine have received extensive research attention. Recently, our laboratory has found that environmental enrichment may decrease the actions of acute nicotine administration on locomotor activity (N.E. Grunberg, personal communication, 2008). No one has examined whether environmental influences alter actions of chronic nicotine administration or cessation. The present series of experiments examine whether environmental enrichment alters effects of icotine administration and cessation on body weight, food consumption, and activity.

Obesity and Body Weight

Obesity is the second leading preventable cause of death within the United States, accounting for roughly 365,000 deaths/year in the U.S. and 17 million deaths/year worldwide. Obesity leads to a variety of chronic health conditions, including cardiovascular disease, diabetes, and metabolic syndrome. Despite the health hazards of obesity, approximately 33% of Americans are obese (Centers for Disease Control and Prevention, 2007c; Centers for Disease Control and Prevention, 2007e; Centers for Disease Control and Prevention, 2007d; Centers for Disease Control and Prevention, 2008; U.S. Department of Health and Human Services, 2000c; U.S. Department of Health and Human Services, 2001c; Grunberg, 1986).

Body weight is influenced by multiple factors: energy intake, energy expenditure, and metabolism (Speakman, 2004). The basic relationship between energy intake, expenditure, and body weight is that the balance of energy intake

and energy expenditure determines body weight. If energy intake exceeds energy expenditure, then body weight increases. If energy intake is less than energy expenditure, then body weight decreases. If energy intake is equal to energy expenditure, body weight remains stable. Several factors contribute to metabolism, including genetics, physical activity, eating patterns, and various drugs. One of these drugs is nicotine. Specifically, nicotine increases metabolism, leading to decreased body weight (Speakman, 2004). Some smokers, particularly female smokers, are afraid to quit smoking because of subsequent weight gain. Although all three factors are related, most individuals focus on energy intake, energy expenditure, or both to alter body weight.

In the United States, the obesity epidemic is a result of both increased energy intake and decreased energy expenditure (Chiolero et al., 2008; Daniels, 2006; Friedman, 2003; Hill et al., 2003; Pi-Sunyer, 2002). In terms of increased energy intake, not only are portion sizes bigger, but individuals are also consuming more high fat foods which have more calories. In terms of decreased energy expenditure, activities of daily living do not require as much physical activity in the past, due in part to technological advances. Many items or products are now powered by battery or electricity, requiring less physical effort to use. In addition, cultural norms regarding physical activity have changed. People drive to most places rather than walking or biking. In fact, people will often drive to the gym to walk or run on a treadmill. Both of these factors, increased energy intake and decreased energy expenditure, have greatly

contributed to the obesity epidemic plaguing America (Chiolero et al., 2008; Daniels, 2006; Friedman, 2003; Hill et al., 2003; Pi-Sunyer, 2002).

As previously mentioned, internal and external factors both contribute to weight. Internal factors include biology (metabolism, genetics, hunger, and satiety), as well as habit, attitudes, beliefs, and values toward weight, eating, and activity. Classical and operant conditioning, boredom, and coping also may contribute to overweight and obesity. Overeating, which contributes to overweight, for example may contribute to anxiety such that anxiety becomes the cue or stimulus for overeating the response. Operant conditioning may occur when an individual is rewarded for overeating or punished for not eating. The taste may be a reward for overeating, for example, especially if it is high fat or high caloric. Children might be punished by their parents for not finishing their dinner, even if they are no longer hungry. Individuals in both scenarios will be more likely to overeat the next time they encounter food, contributing to overweight. External factors also are large contributors to overweight and obesity. As previously mentioned, cultural values are one type of external factor that may contribute to overweight. The availability of foods, including types of foods as well as costs of food, may explain some socioeconomic status (SES) differences in prevalence of overweight. Investigating how multiple factors contribute to body weight will be beneficial in that it may lead to better interventions for weight challenges, as well as preventive methods. One factor, physical activity, is of particular interest.

Biological, behavioral, and environmental variables relevant to obesity and body weight have received extensive research attention. For example, nicotine is well known to reduce body weight. Recently, our laboratory has found that environmental enrichment also may decrease body weight (Shafer, 2006; Tomchesson, 2004, 2006). No one has examined whether environmental influences alter actions of chronic nicotine administration or cessation on body weight.

Physical Activity

Over 50% of American adults do not engage in recommended levels of physical activity (Centers for Disease Control and Prevention, 2008d; U.S. Department of Health and Human Services, 1996b; U.S. Department of Health and Human Services, 2000b; U.S. Department of Health and Human Services, 2001d). Further, approximately 25% of the American population is sedentary. Physical activity decreases with age, is less common among women compared with men, and is less common among low socioeconomic status individuals compared with high socioeconomic status individuals. This alarming lack of physical activity has contributed to the health crisis of obesity in this country. Physical activity is necessary to health and lack of physical activity leads to a variety of chronic health conditions, including cardiovascular disease, obesity, diabetes, and metabolic syndrome. Despite the health benefits of physical activity, and the health risks of inactivity, a large majority of the U.S. population does not engage in physical activity on a regular basis (U.S. Department of

Health and Human Services, 1996a; U.S. Department of Health and Human Services, 2000a; U.S. Department of Health and Human Services, 2001a).

Physical activity is defined as any bodily movement produced by skeletal muscle which results in energy expenditure (Centers for Disease Control and Prevention, 2008c). Exercise is a subset of physical activity. It is defined as physical activity that is both planned and structured with the intention to improve or maintain physical fitness (Centers for Disease Control and Prevention, 2008c). Physical fitness determines a person's ability to perform physical activities and is determined by a combination of regular activity and genetically inherited ability (Centers for Disease Control and Prevention, 2008c). Physical fitness has five components: cardiorespiratory endurance, muscular strength, muscular endurance, flexibility, and body composition (Centers for Disease Control and Prevention, 2008c). The American College of Sports Medicine (ACSM) regularly produces recommendations for physical activity – the recommendations differ based on the intent of physical activity, whether for health or for weight loss. The ACSM recommends that individuals engage in regular moderate physical activity on most days of the week for health purposes. Moderate physical activity can be defined in several ways: some increase in breathing or heart rate, a perceived exertion of 11 to 14 on the Borg scale, 3 to 6 METs, or any activity that burns 3.5 to 7 calories per minute (kcal/min). For weight loss, individuals should engage in 60-90 minutes of regular moderate physical activity on most days of the week (Centers for Disease Control and Prevention, 2008b).

Physical inactivity is maintained by psychological, social, and environmental factors. Lack of self-efficacy for physical activity, decreased need for physical activity due to technological changes, and other psychological and social variables also reinforce physical inactivity in humans. Physical activity is related to both nicotine and body weight. Nicotine increases physical activity, but this effect may be environmentally-dependent (Faraday, Elliott, Phillips, & Grunberg, 2003). Physical activity, as previously mentioned, results in energy expenditure, weighting the energy balance towards weight loss. All three of these factors, then, are related. Given the difficulty inherent in changing just one of these behaviors, it may be overwhelming to ask an individual to change all three at once. Although it is unclear whether attempting sequential or collective multiple behavior change is more effective, feasible, etc, focusing on external factors may help the process.

Behavioral and environmental variables relevant to physical activity have received extensive research attention. Recently, our laboratory has found that environmental enrichment decreases open field activity but increased activity in the home cage (Tomchesson, 2006). No one has examined whether environmental enrichment alters voluntary exercise in a novel environment. Further, no one has examined whether environmental influences alter actions of chronic nicotine administration or cessation on physical activity.

Environment

The environment influences all health behavior variables, including tobacco use, obesity, and physical activity. Altering the environment, therefore, may have a substantial effect on these particular health behaviors. The environment also influences biological variables, as noted by Charles Darwin (1875) when comparing the brain sizes of domestic rabbits to wild rabbits. Specifically, Darwin (1875) found that the brains of domestic rabbits were considerably smaller than those of wild rabbits, which he attributed to the "deprive environments" of domestic rabbits. Donald Hebb (1947) also noted that enhanced environments resulted in improved learning in laboratory rats. He hypothesized that these enhanced environments caused structural changes in the brain which then improved learning abilities. Approximately twenty years later, Mark Rosenzweig (1966) introduced the classic paradigm of environmental enrichment and found that enrichment does indeed cause structural brain changes, supporting Hebb's hypothesis.

The classic enrichment paradigm involves two forms of enrichment: physical and social. Physical enrichment involves adding objects to the home cage to allow for tactile stimulation and physical activity (Rosenzweig et al., 1972b; Woodcock & Richardson, 2000b; Woodcock & Richardson, 2000a). Social enrichment involves housing animals in groups of two or more to allow for social interaction. Standard housing conditions in rats involve limiting one animal to a cage without objects (Varty et al., 2000).

The environment has numerous effects on biological and behavioral factors. Biological effects include increased neurogenesis, decreased food consumption, and decreased body weight (Diamond et al., 1972; Fernandez-Teruel et al., 2002; Johansson, 2003; Kleim et al., 2003; Kramer et al., 2002; Mohammed et al., 2002; Rosenzweig et al., 1972a; Sutoo & Akiyama, 2003; Tomchesson, 2004, 2006; Van de Weerd et al., 2002). Behavioral effects include improved attention, improved performance on learning and memory tasks, and decreased anxiety (Benaroya-Milshtein et al., 2004; Chapillon et al., 1999; Daniel et al., 1999; Elliott & Grunberg, 2005; Friske & Gammie, 2005; Pham et al., 1999; Pietropaolo et al., 2004; Schrijver et al., 2002; Tomchesson, 2004, 2006; Williams et al., 2001; Zimmermann et al., 2001).

Our laboratory recently has found that environmental enrichment also alters activity. Enrichment increases home cage activity, especially when large cages are provided that allow for markedly greater movement (Tomchesson, 2006). In contrast, enrichment decreases open field activity (Shafer, 2006; Tomchesson, 2006), presumably because of improved learning (i.e., habituation to the open field).

Few researchers have examined the effects of environmental enrichment on the actions of drugs. Bardo and colleagues (1995) reported that environmental enrichment attenuated amphetamine's effects on locomotor sensitization. Green and colleagues (2003) also reported that environmental enrichment decreases the effects of acute and repeated acute injections of nicotine on activity. No one has examined whether environmental influences

alter actions of chronic nicotine administration or cessation on voluntary exercise. Chronic nicotine administration provides a more accurate model of human tobacco use. Investigating the impact of environmental influences on chronic nicotine administration also provides a more accurate model of what occurs in the human condition. Specifically, human tobacco use occurs in the context of physical and social environmental influences. Further, the effects of exercise on tobacco use and cessation have been explored to some degree, but research literature on the effects of tobacco use and cessation on exercise is lacking. deRuiter and Faulkner (2006) have proposed the investigation of physical activity as a harm-reduction strategy for individuals who continue to use tobacco products. Kinnunen et al. (2008) reported that exercise helps prevent relapse during tobacco cessation. One of the few studies in the literature reported that transdermal nicotine has beneficial effects on exercise endurance (Mundel & Jones, 2006), which may encourage individuals to either begin or to maintain tobacco use despite the numerous negative health consequences. It is, therefore, important to investigate how environmental influences and nicotine administration and cessation may impact voluntary exercise in addition to other forms of physical activity.

These same principles of environmental changes may be used to influence health-related behaviors. If we understand how environmental factors influence behavior, large scale interventions and preventions may be implemented. These large scale treatments would reach a larger proportion of the population and may be more effective, particularly when combined with

individual treatment. Before such studies in humans may be attempted, it would be prudent to investigate how environmental factors influence health behaviors in animals.

Benefits of Animal Models

Previous studies have used animal models to investigate environmental factors' influence on health behaviors. Animal models allow increased experimental control and the ability to conduct experiments that would not be ethical in human research. In contrast, animal models lack face validity and lack unique aspects of the human experience. The benefits of using an animal model, however, outweigh the disadvantages in this experiment.

Overview of Experiments

This master's thesis research includes three experiments. Experiment I examined the effects of environmental enrichment on body weight, food consumption, and activity in male Sprague Dawley rats. Experiment IIa examined the effects of environmental enrichment and chronic nicotine administration on body weight, food consumption, and activity. Experiment IIb examined the effects on environmental enrichment and chronic nicotine cessation on body weight, food consumption, and activity.

Experiment I built upon previous experiments in the Grunberg lab which examined the effects of environmental enrichment on body weight, food consumption, open field activity, and home cage activity (Tomchesson, 2004, 2006). Experiment I also extended previous experiments by adding another dependent variable: voluntary exercise. Experiment II extended previous

experiments which examined the effects of environmental enrichment on acute injections of nicotine on open field activity, by examining chronic administration of nicotine and measuring additional dependent variables. Shafer's (2006) work on the effects of environmental enrichment on nicotine withdrawal also laid the foundation for part b of Experiment II.

Experiment I was necessary to establish baseline measures of the dependent variables (body weight, food consumption, and activity), as well as to allow the rats to mature to adulthood. Experiment II introduced one of the key independent variables, nicotine. Experiment II was divided into two parts, a and b, to separate the effects of chronic nicotine administration and cessation.

This master's thesis discusses each experiment separately and in detail, including methods, results, and a brief discussion. After each experiment is reviewed, a general discussion is provided to synthesize the findings from the three experiments. This master's thesis then discusses clinical implications, limitations, and future directions.

Experiment I

Overview

This experiment was designed to evaluate effects of housing condition on physical activity, food consumption, and body weight of rats. There were four different housing conditions that manipulated the social and physical environment. Physical activity was measured in three different ways: home cage activity, movement in an open field locomotor chamber, and activity in exercise wheels. This experiment lasted five weeks. The experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC) and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Institutes of Health [NIH], 1996).

Hypotheses

1. Enriched housing will decrease body weight, with super-enrichment having the greatest effects. This hypothesis is based on the findings of Shafer (2006) and Tomchesson (2006): both found that super-enrichment attenuates body weight gain.
2. Enriched housing will have minimal effects on food consumption. This hypothesis is based on the findings of Shafer (2006) and Tomchesson (2006): both found that enrichment had minimal effects on food consumption.
3. Enriched housing will differentially affect activity, resulting in:
    a. decreased open field activity, with greater enrichment having greater effects (NE<PE=SE<Sup). This hypothesis is based on the findings of

Shafer (2006) and Tomchesson (2006): both found that enrichment, especially super-enrichment, decreased open field activity.

b. increased home cage activity, with super-enrichment having the greatest effects. This hypothesis is based on the findings of Tomchesson (2006), who found that super-enrichment resulted in increased home cage activity.

No hypothesis was made for the effects of enriched housing on voluntary exercise because of the lack of discussion in the literature.

Subjects

Subjects were 64 male Sprague Dawley rats (Charles River Laboratories). They arrived at 21 days of age weighing between 40 and 50 grams and were immediately placed into experimental housing conditions.

Housing

A hardwood chip bedding (Pine-Dri) was used in all cages. All subjects had continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. The housing room was maintained at approximately 23 degrees C and approximately 50% relative humidity on a 12-hour reversed light/dark cycle (lights off at 0600 hours).

Upon arrival, subjects were sequentially assigned to one of four housing conditions: (1) isolated or non-enriched (NE); (2) physically-enriched (PE); (3) socially-enriched (SE); or (4) super-enriched (Sup; *See Appendix A for pictures*). In the NE condition subjects were housed singly in standard polycarbonate rat cages (40 x 20 x 20 cm) without physical objects or toys. In the PE condition

subjects were housed singly in standard polycarbonate rat cages with a water bottle and food and two toys (e.g., hard plastic balls, tunnels, cars). The toys were changed twice weekly and replaced with new toys that were dissimilar from the old to maintain novelty. In the SE condition two subjects were housed in standard polycarbonate cages with a water bottle and food but no toys. In the SUPER condition eight subjects were housed in a three-level galvanized steel cage (76 x 61 x 137 cm) with several water bottles and food cups and eight toys. The toys were changed twice weekly and were distributed in the Super cage with three toys on the top level, three toys on the middle level, and two toys on the bottom level. The polycarbonate cages were changed twice weekly and the bedding of the Super cages also were changed twice weekly. These housing conditions were based on previous reports (Elliott, 2004; Elliott & Grunberg, 2005; Tomchesson, 2004, 2006; Shafer, 2006).

Procedures

On the first day of the experiment, subjects were assigned to one of the four housing conditions. On each of the subsequent three days, each subject was briefly gentled (by handling about 3 minutes each) to attenuate or prevent stress responses due to handling that was required to measure body weight and to place animals into the open field chambers and exercise apparatus.

Throughout the experiment, food consumption (FC) and home cage activity (HCA) were measured every other day. Open field activity (OF) was measured twice during the experiment (two weeks apart). Activity in exercise wheels (EX) was measured twice during the experiment (during the weeks when

OF was not measured). Body weight (BW) was measured weekly before either the OF or EX measure. Due to logistical considerations, not all animals' open field activity and voluntary exercise were measured on the same day. Animals were split into two cohorts for open field activity and four cohorts for voluntary exercise an equal number of animals from each housing condition were evaluated during each measurement. As body weights were measured before OF or EX, body weights for the different cohorts were measured on different days. These differences are reflected in the timeline below, such that BW (all) indicates that all animals' body weights were measured on a given day, BW (1/2) and OF (1/2) indicate that half of the animals' body weights and open field activity were measured on a given day, and BW (1/4) and EX (1/4) indicate that a quarter of the animals' body weights and voluntary exercise were measured on a given day.

Experiment I Timeline	
Day	**Measures taken**
1	Rats arrive, assign to housing, FC
2	Gentling, BW (all)
3	Gentling, FC, HCA
4	Gentling, BW (all)
5	FC, HCA
6	
7	FC, HCA
8	BW (1/2), OF (1/2), Change T+C
9	FC, HCA
10	
11	BW (1/2), OF (1/2), FC, HCA, Change T+C
12	
13	FC, HCA
14	
15	BW (1/4), Ex (1/4), FC, HCA, Change T+C
16	BW (1/4), Ex (1/4)
17	BW (1/4), Ex (1/4), FC, HCA
18	BW (1/4), Ex (1/4), Change T+C

19	FC, HCA
20	
21	FC, HCA
22	BW (1/2), OF (1/2), Change T+C
23	FC, HCA
24	
25	BW (1/2), OF (1/2), FC, HCA, Change T+C
26	
27	FC, HCA
28	
29	BW (1/4), Ex (1/4), FC, HCA, Change T+C
30	BW (1/4), Ex (1/4)
31	BW (1/4), Ex (1/4), FC, HCA
32	BW (1/4), Ex (1/4), Change T+C
33	FC, HCA
34	
35	FC, HCA
36	
37	FC, HCA

Body weight (BW). BW measurements were taken once per week using a Sartorius electronic balance programmed to provide the mean weight of multiple measurements taken in rapid succession in order to avoid movement artifacts. BW measurements were taken on the same day as OF and Ex measurements to minimize handling.

Food consumption (FC). FC was measured every other day throughout the experiment. Cage lids with food were weighed and the amount of food was calculated based on the change in weight. For animals that were group housed (i.e., SE and SUPER conditions), the change in weight was divided by the number of animals within the cage. When new food was added, the lids plus food pellets were weighed and recorded. In the SUPER condition, the food cups plus food were weighed.

Open field activity (OF). OF was assessed once every other week for a total of two times during this experiment using an Omnitech/Accuscan Electronics Digiscan infrared photocell system (Omnitech Electronics, Columbus, OH) in a dedicated procedure room close to but separate from the housing room. The temperature and humidity of this procedure room was similar to the housing room. Red overhead lights were on when subjects were placed into and removed from the locomotor chambers. The room was dark during testing. Animals were placed singly in a 40 x 40 x 30 cm clear Plexiglas chamber for a period of one hour. The chambers had Plexiglas lids with ventilation holes (3.5cm diameter) to prevent escape. A photocell array measured horizontal locomotor activity using 16 pairs of infrared photocells located every 2.5 cm from side-to-side and 16 pairs of infrared photocells located front-to-back in a plane 2 cm above the floor of the arena. A second side-to-side array of 16 pairs of additional photocells located 10.5 cm above the arena floor measured vertical activity. Data were automatically gathered and transmitted to a computer via an Omnitech Model DCM-I-BBU analyzer in 12 5-minute bins. The interfaced software generates 21 sub-variables. Horizontal activity, vertical activity, and center time were analyzed.

Home cage activity (HCA). HCA was rated by two experimenters in the middle of the day every other day throughout the experiment. The experimenters quietly entered the darkened room and turned on an overhead red light (so that the room was dimly lit in red light to allow experimenters, but not albino rats, to see). The experimenters waited and talked quietly to allow rats to acclimate to

the presence of experimenters. Then, each experimenter independently observed the movement of all of the subjects in one housing condition for 1 minute. Each experimenter then used a 7-point Likert format to rate the number of animals moving, the amount of activity for the majority of group members, and the level of effort of activity. In addition, experimenters recorded the type of activity and the number of animals engaged in each type of activity. This procedure was repeated 39 times throughout this experiment. The order of conditions observed changed with each observation period. The procedure was based on Tomchesson (2006). (See Appendix B for a copy of the HCA rating sheet.) Experimenters were trained in this method by experienced lab members who had previously used the HCA rating sheet. During training, the experienced lab members explained the purpose and procedure. The trainers and trainees then practiced the procedure until the ratings were consistent. Any differences were discussed by the trainers and trainees until an agreement was reached. After approximately eight practice sessions, the trainees were considered proficient in this measurement procedure. Past research in the Grunberg lab has demonstrated an inter-rater reliability score of at least 80% in this measure.

Activity in exercise wheels (Ex). Activity in exercise wheels was measured every other week for a total of two times during this experiment in Med Associates activity wheels (Med Associates, Inc., St. Albans, VT). The equipment was in a dedicated procedure room that was separate from but nearby the housing and open field activity rooms. The temperature, humidity, and lighting conditions were the same as those conditions in the open field

activity room. The equipment consisted of eight activity wheels (35.6 cm diameter) consisting of stainless steel grid rods (4.8 mm diameter) spaced 1.6 cm apart. Each activity wheel was connected to a separate plastic cage (48.26 cm L x 26.67 cm W x 20.32 cm D) with a stainless steel wire cover. Each cage has a 7.2 cm W x 10.2 cm H opening that allows voluntary access to the running wheel or cage. Each activity wheel had 12 grams of drag. Rats were placed singly into the plastic cages and were allowed access to the exercise wheels. Revolutions of each activity wheel were recorded automatically on a dedicate computer that was interfaced with the activity wheels during a two hour access period. The data (number of quarter revolutions of the activity wheel) were electronically recorded in 120 1-minute bins.

Data Analytic Strategy for Experiment I

Subjects were randomly assigned to housing conditions upon arrival. Although analyses of variance (ANOVA) were used for all data analyses, the particular version of ANOVA varied based on the dependent variable under study.

Body weight and food consumption were analyzed using repeated-measures ANOVAs to assess over time throughout the experiment. Any significant main effects or interactions were examined using separate ANOVAs following the procedures of Keppel (1991). If there was a significant effect, then Tukey HSD *post-hoc* analyses were performed. F values, degrees of freedom and p values for analyses in Experiment I are provided in Appendix A.

Open-field activity was analyzed using repeated-measures ANOVAs to examine the effects of enrichment on locomotor activity. For all open-field activity analyses, enrichment was the between-subjects factor and time was the within-subjects factor. Three separate repeated-measures ANOVAs were computed for each of three different types of activity recorded in the open-field chambers (i.e., horizontal activity, vertical activity, and center time). Although all three measures are conceptually related and could be analyzed with a multivariate ANOVA, the changes in these types of activity over time were of greater interest than how the three were related within a single open-field activity session. In addition, center time ratios were computed and analyzed with a repeated-measures ANOVA. Within-session open-field activity was also analyzed using a repeated-measures ANOVA. If the initial analyses indicated significant between-subjects effects, then repeated-measures ANOVA were performed separately for each of the open-field trials.

Home cage activity was analyzed using repeated-measures ANOVAs for the pre-drug phase of the experiment. For all analyses, enrichment was the between-subjects factor and time was the within-subjects factor. Three separate repeated-measures ANOVAs were computed for each of three different types of home cage activity (i.e., number of animals moving, amount of activity, and effort of activity). Although all three measures are conceptually related and could be analyzed with a multivariate ANOVA, the changes in these types of activity over time were of greater interest than how the three were related within a single week of home cage activity. Home cage activity was not analyzed during the drug and

post-drug phases due to inherent limitations of the measure. The home cage activity measurement was based on the activity of each housing condition as a whole. The measurement did not distinguish between saline and nicotine animals within each housing condition and any differences between housing conditions would be confounded by the drug effects.

Exercise was analyzed with a repeated-measures ANOVA. Enrichment was the between-subjects factor and time was the within-subjects factor. Latency to start movement in the activity wheel was analyzed similarly. The number of full wheel rotations during the first minute of activity was also analyzed using an ANOVA. Similarly to open-field activity, within-session exercise was analyzed using a repeated-measures ANOVA. Enrichment was the between-subjects factor and time was the within-subjects factor. If the initial analyses indicated significant between-subjects effects, then repeated-measures ANOVA were performed separately for each of the exercise trials.

Several strategies were used to minimize the probability of Type I and Type II error. First, only if overall analyses revealed a significant main effect or interaction were subsequent analyses performed. This strategy reduces the number of statistical tests performed (Keppel, 1991; Cohen & Cohen, 1983). All tests were two-tailed with significance determined by $p \leq 0.05$. In addition, the experiment was designed to provide adequate power (0.80). Type II error is minimized when sample size supports adequate power (Keppel, 1991).

Data were excluded from the analyses only if two criteria were met: (1) data points were more than three standard deviations from the mean of the

experimental condition corresponding to those data, and (2) data were inconsistent with the subject's scores over time. To determine inconsistency, each datum was compared with the subject's previous and next datum for that particular subject. If disparate, the data were excluded from analyses. Thirty-two data points of 1,996 total data points were excluded from analyses, all of which met the above criteria and were from the food consumption data set. Specifically for food consumption, if a datum was two times greater or less than the previous and next datum, or was a negative change score (i.e., the food weight had increased from one measurement to the next), it was considered inconsistent. If the datum was also three standard deviations from the mean, , then it was excluded from data analyses.

Results for Experiment I

Body weight (see Figure 1). Body weight was measured once weekly throughout the experiment. Repeated-measures ANOVA revealed a main effect for time ($F [4, 240] = 2504.784$, $p < 0.001$) and a time by enrichment interaction ($F [12, 240] = 2.148$, $p < 0.05$), indicating that enriched and non-enriched animals both gained weight over the course of the experiment (as expected given their young age at the start of the experiment), but that animals in the different housing conditions gained weight differentially. Animals began at approximately the same body weight, but the super-enriched condition resulted in attenuated body weight gain for weeks 2, 3, and 4. This difference disappeared in week 5. A one-way ANOVA on the third week revealed a main effect for enrichment ($F [3, 64] = 7.453$, $p < 0.001$). Specifically, super-enriched weighed less than non-enriched and physically-enriched animals (NE=PE>SUP). Social-enriched animals did not differ significantly from any of the other groups.

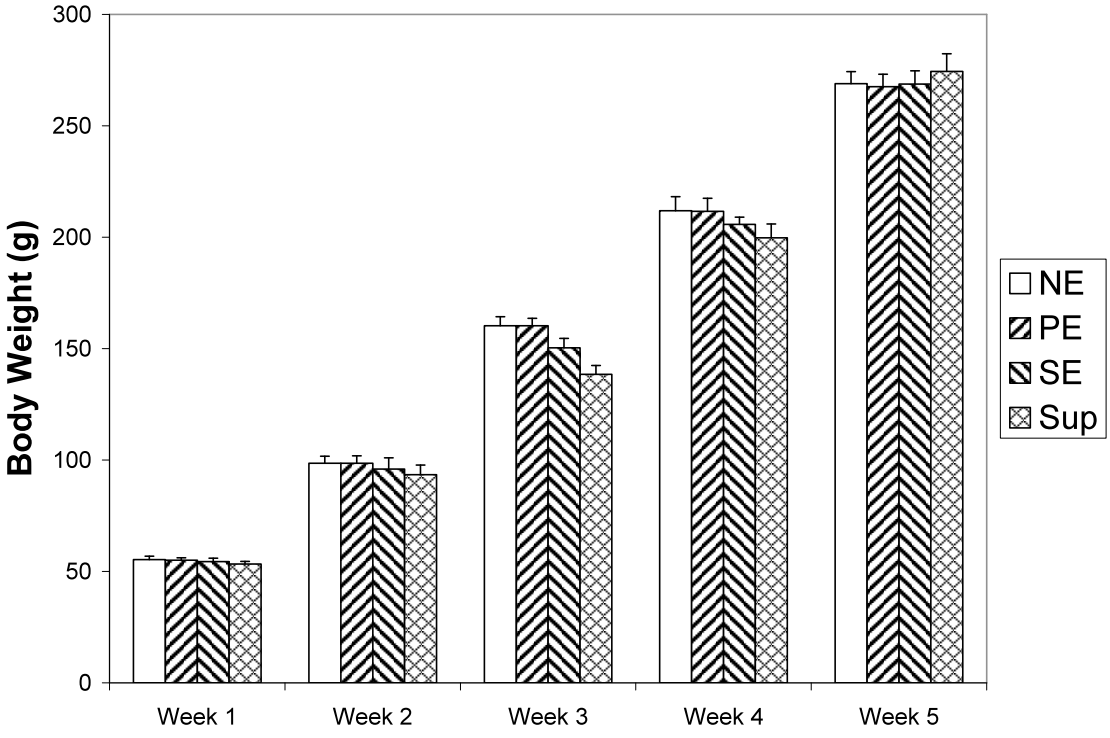

Figure 1. Mean body weights (SEM) of male Sprague Dawley rats in four different housing conditions (NE = non-enriched; PE = physically-enriched; SE = socially-enriched; Sup = super-enriched)

Food consumption (see Figure 2). Food consumption was measured every other day throughout the experiment. The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. A repeated-measures ANOVA revealed a main effect for time ($F [1.967, 114.101] = 2534.69$, $p < 0.001$) and a time by housing interaction ($F [5.902, 114.101] = 7.353$, $p < 0.001$), but no main effect for housing. These analyses indicate that all animals consumed more food over

time, but that animals in the different housing conditions increased their rate of food consumption differentially. Specifically, the non-enriched, physically-enriched, and socially-enriched animals all appeared to increase their food consumption at similar rates, but the super-enriched animals appeared to increase their food consumption at a greater rate than did the animals in the other housing condition. Subsequent analyses revealed main effects for housing during certain weeks, but not throughout the experiment. Main effects for housing were detected for weeks 1, 2, and 5.

During week 1, housing conditions differed significantly ($F [1, 60] = 11.815$, $p < 0.001$), a difference that was not present at the start of the experiment. Specifically, both the NE and PE conditions ate significantly more food than did both the SE and Sup conditions (NE=PE>SE=SUP). During week 2, housing conditions also differed significantly ($F [1, 58] = 2.955$, $p < 0.05$), but *Post hoc* analyses revealed that only the NE condition ate significantly more food than the SE condition (NE>SE). Other differences between the conditions had disappeared. The differences in food consumption reappeared during week 5 of the experiment ($F [1, 59] = 4.136$, $p < 0.01$), although in a different condition. Both the NE and the SE conditions ate significantly less food than the Sup condition (NE=SE<SUP). Not only did the differences between housing conditions disappear at week 4, but they reversed at week 5.

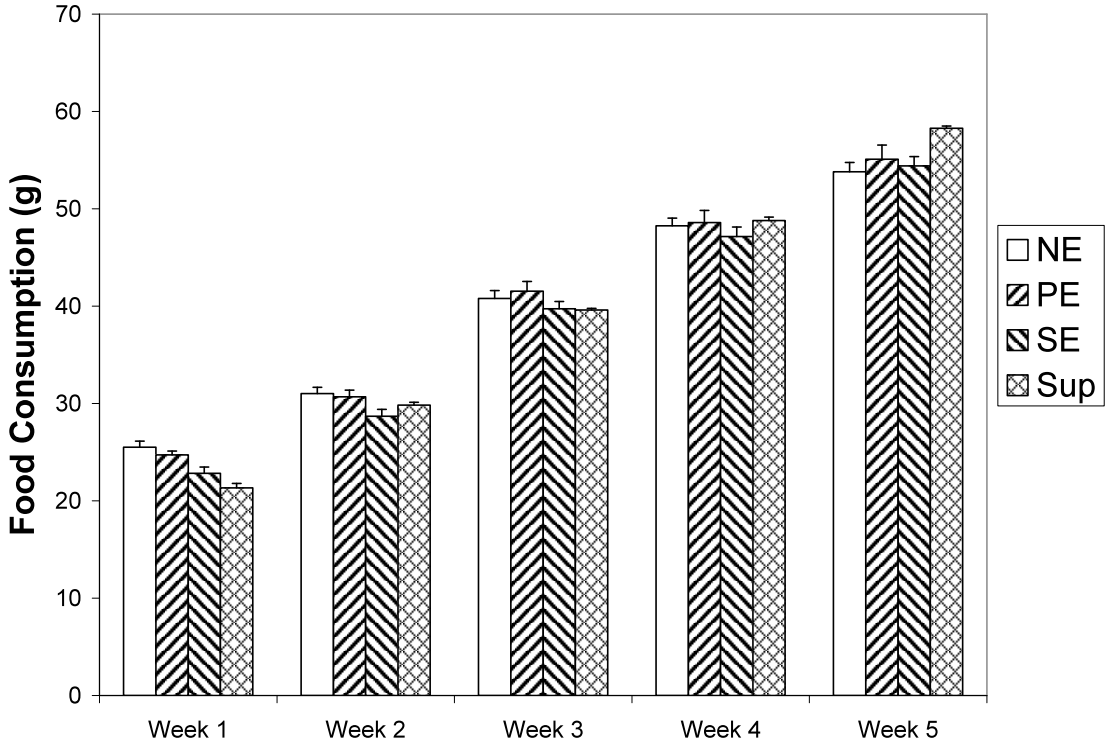

Figure 2. Mean food consumption (± SEM) of male, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

Open field activity (see Figures 3-4). Locomotor activity was measured in the open field chambers for 60 minutes, twice during each phase of the experiment. Overall, horizontal activity provides an index of overall activity and health, as well as an index of reward. Horizontal activity changes within a 60 minute session provide an index of learning and habituation. Vertical activity provides an index of exploration and/or escape. Changes in center time (relative to total time moving) provide an index of changes in anxiety.

The repeated-measures ANOVA for horizontal activity revealed a main effect for housing ($F [3, 60] = 28.333$, $p < 0.001$), but no main effect for time and no time by housing interaction. *Post hoc* analyses revealed that non-enriched animals had the greatest amount of horizontal activity, followed by physically- and socially-enriched animals, followed by super-enriched animals (NE>PE=SE>Sup).

The repeated-measures ANOVA for vertical activity revealed no significant effects. The repeated-measures ANOVA for center time activity revealed a main effect for time ($F [1, 60] = 12.026$, $p < 0.001$) and a main effect for housing ($F [3, 60] = 3.190$, $p < 0.05$), but no time by housing interaction. Animals increased center time activity from the first open field measurement to the second open field measurement, indicating decreased anxiety levels. *Post hoc* analyses revealed that socially-enriched animals had greater center time activity than did super-enriched animals (SE>Sup).

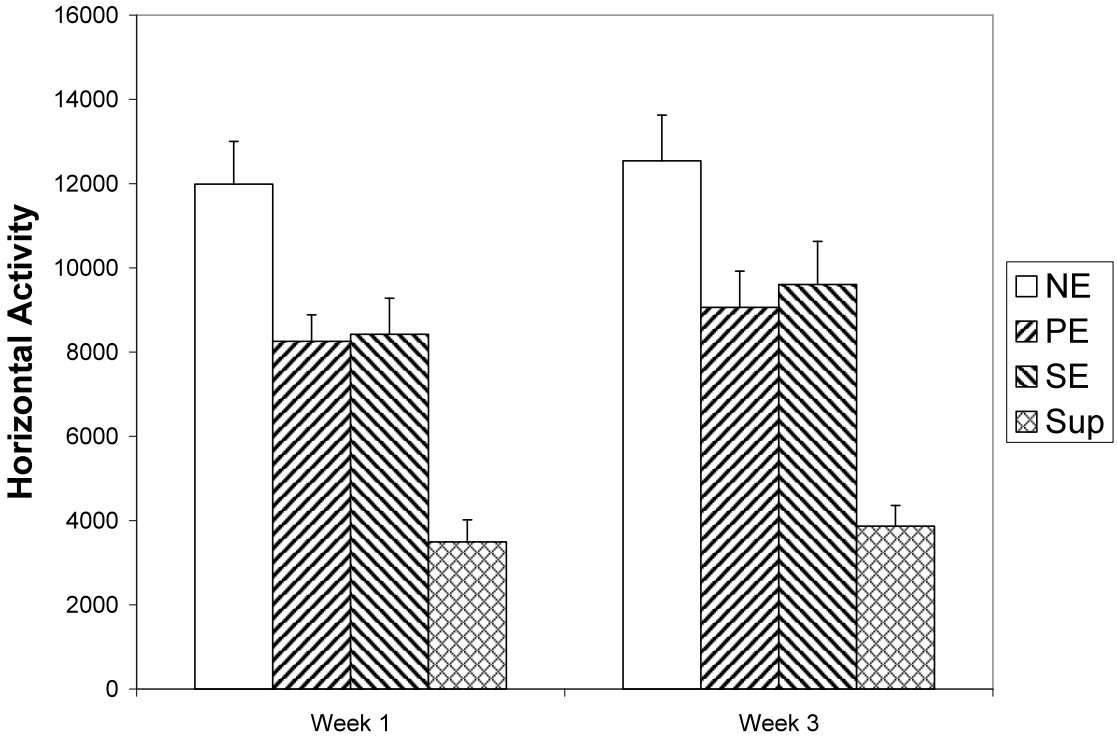

Figure 3. Mean open field horizontal activity (± SEM) of male, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The repeated-measures ANOVA for the first within-session open field activity also yielded statistically significant results. The analysis revealed a main effect for time ($F [4.171, 250.333] = 198.759$, $p < 0.001$), a main effect for housing condition ($F [3, 60] = 20.982$, $p < 0.001$), and an interaction between the two ($F [12.512, 250.233] = 9.733$, $p < 0.001$). Horizontal activity decreased over time, but more quickly for the super-enriched condition than the other housing

conditions. *Post-hoc* analyses revealed that the non-enriched condition had greater amounts of activity than the physically-enriched, socially-enriched, and super-enriched conditions (NE>PE=SE>Sup). The physically-enriched and socially-enriched conditions also had greater amounts of activity than the super-enriched condition (PE=SE>Sup).

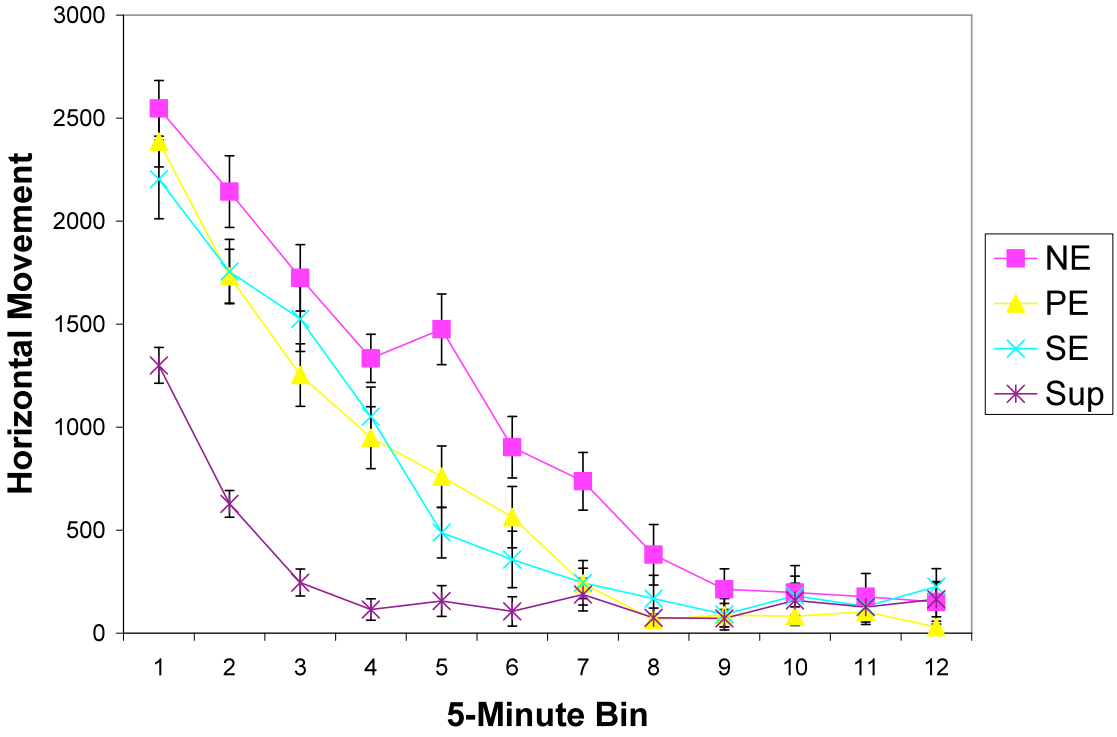

Figure 4. Mean within-session week one open field horizontal activity (± SEM) of male, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The

repeated-measures ANOVA for the second within-session open field activity also yielded statistically significant results. The analysis revealed a main effect for time ($F [5.316, 318.939] = 202.089$, $p < 0.001$), a main effect for housing condition ($F [3, 60] = 16.293$, $p < 0.001$), and an interaction between the two ($F [15.947, 318.939] = 5.878$, $p < 0.001$). Horizontal activity decreased over time, but more quickly for the super-enriched condition than the other housing conditions. *Post-hoc* analyses revealed that the non-enriched condition had greater amounts of activity than the physically-enriched and super-enriched conditions (NE>PE>Sup). The physically-enriched and socially-enriched conditions also had greater amounts of activity than the super-enriched condition (PE=SE>Sup).

Home cage activity (see Figure 5). Home cage activity was measured every other day by two independent raters each time. Home cage activity provides a unique opportunity to observe the animals in their home environment as opposed to other measures which entail observation in a novel environment (i.e., open field activity and exercise). Home cage activity has three subparts targeted to quantify three different aspects of activity: number of animals moving, amount of activity, and effort of activity. Observations from each week of the experiment were compiled and analyzed with separate repeated-measures ANOVA for each aspect of activity.

The repeated-measures ANOVA for number of animals moving revealed a main effect for time ($F [4, 72] = 7.420$, $p < 0.001$) and a main effect for housing ($F [3, 18] = 8.365$, $p < 0.001$), but no time by housing interaction. The number of

animals moving changed from week to week, such that it increased from week one to week two, decreased from week two to week three, increased from week three to week four, and decreased from week four to week five. *Post hoc* analyses reveal that greater numbers of animals moved in the socially-enriched condition than in the super-enriched condition.

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The repeated-measures ANOVA for amount of activity revealed a main effect for time ($F [2.074, 39.401] = 3.432$, $p < 0.05$) and a main effect for housing ($F [3, 19] = 6.971$, $p < 0.01$), but no time by housing interaction. The amount of activity changed from week to week in a pattern similar to that of number of animals moving, such that it increased from week one to week two, decreased from week two to week three, increased from week three to week four, and decreased from week four to week five. *Post hoc* analyses revealed that socially-enriched animals engaged in greater amounts of activity than did non-enriched, physically-enriched, and super-enriched animals (SE>NE=PE=Sup).

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The repeated-measures ANOVA for effort of activity revealed a main effect for housing ($F [3, 19] = 5.840$, $p < 0.01$), but no main effect for time and no time by housing interaction. *Post hoc* analyses revealed that socially-enriched animals had greater effort of activity than did non-enriched and super-enriched animals

(SE>NE=Sup). Physically enriched animals did not differ significantly from any of the other three housing groups.

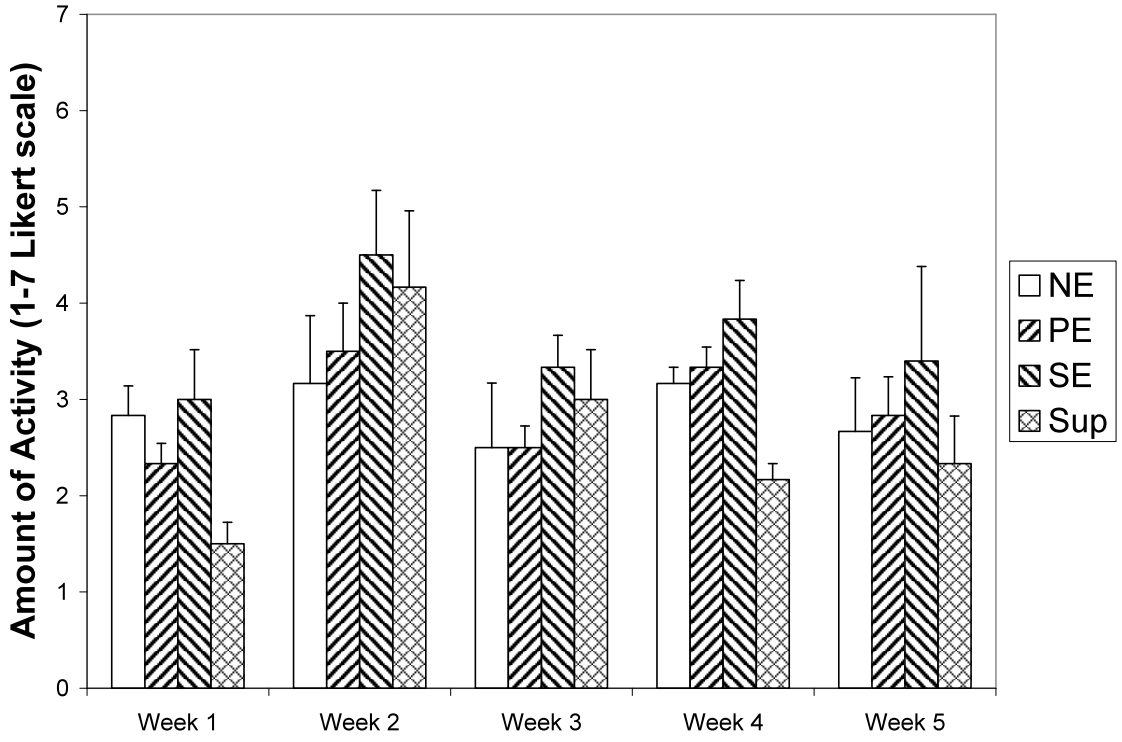

Figure 5. Mean amount of activity within the home cage (± SEM) of male, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

Exercise (*see Figures 6-7).* Exercise was measured in activity wheels attached to a plastic standard size cage for 120 minutes, twice during Experiment I. Exercise was considered voluntary as animals had free access to the activity wheels and could move freely between the activity wheel and the plastic cage. Bedding was added to the plastic cage to make it more comparable to the home cage. Total number of full revolutions were recorded electronically and indicate

total amount of voluntary exercise. Latency to start was recorded as a measure of exploration, such that greater latency indicated less exploration. Number of full revolutions during the first minute of activity was also calculated to indicate amount of voluntary exercise not influenced by habituation or by learning.

A repeated-measures ANOVA revealed a main effect for housing ($F [3, 60] = 10.118$, $p < 0.001$). *Post hoc* analyses revealed that non-enriched, physically-enriched, and socially-enriched animals all engaged in greater amounts of exercise than did super-enriched animals (NE>Sup; PE>SE>Sup). Physically-enriched animals also engaged in greater amounts of exercise than socially-enriched animals (PE>SE).

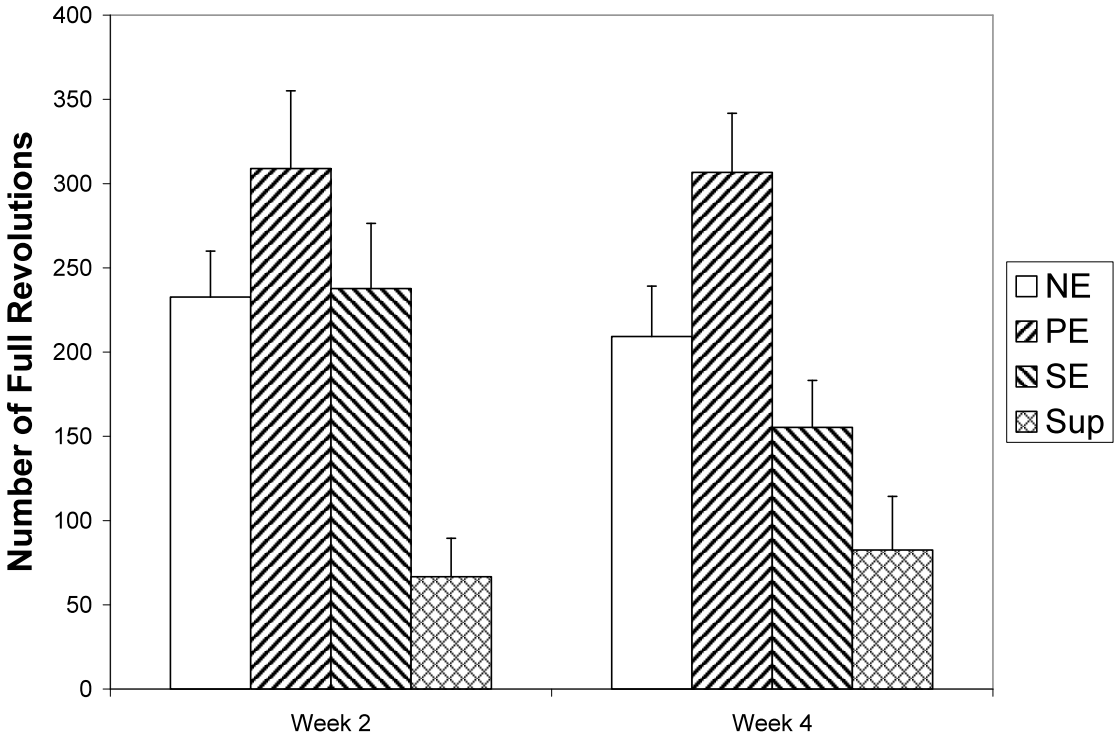

Figure 6. Mean voluntary exercise (± SEM) of male, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. Within-session repeated-measures ANOVA during the first exercise measurement revealed a main effect for housing ($F [3, 60] = 8.616$, $p < 0.001$), a main effect for time ($F [1.185, 71.083] = 143.932$, $p < 0.001$), and an interaction between the two ($F [3.554, 71.083] = 8.183$, $p < 0.001$). In terms of the main effect for housing, *Post hoc* analyses revealed that non-enriched, physically-enriched, and socially-enriched animals all engaged in greater amounts of voluntary exercise than did

super-enriched animals (NE=PE=SE>Sup). In regard to the main effect for time, exercise decreased over time for all groups. In terms of the interaction, the super-enriched animals decreased exercise over time more quickly than the other three groups.

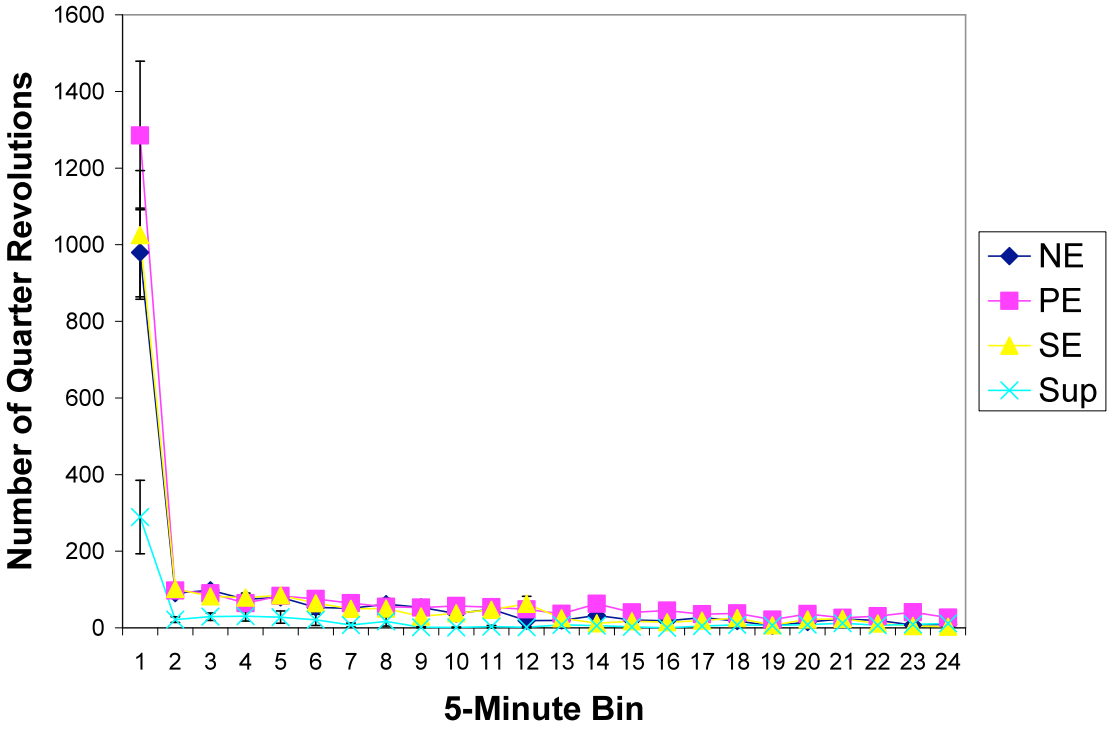

Figure 7. Mean within-session week two voluntary exercise (± SEM) of male, Sprague Dawley rats in four different housing conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. Within-session repeated-measures ANOVA during the second exercise measurement

revealed a main effect for housing ($F [3, 60] = 9.175$, $p < 0.001$), a main effect for time ($F [1.211, 72.633] = 138.587$, $p < 0.001$), and an interaction between the two ($F [3.632, 72.633] = 8.434$, $p < 0.001$). In terms of the main effect for housing, *post hoc* analyses revealed that non-enriched and physically-enriched animals engaged in greater amounts of voluntary exercise than did super-enriched animals (NE=PE>Sup). Physically-enriched animals also engaged in greater amounts of voluntary exercise than did socially-enriched animals (PE>SE). In regard to the main effect for time, exercise decreased over time for all groups. In terms of the interaction, the super-enriched animals decreased exercise over time more quickly than the other groups of animals.

A repeated-measures ANOVA for latency to start indicated a main effect for time ($F[1, 60] = 6.870$, $p < 0.05$), but no main effect for housing condition and no interaction effect. Specifically, during the second exercise measurement, animals had longer latency to start running on the activity wheel than during the first exercise measurement.

A repeated-measures ANOVA for number of full revolutions during the first minute of activity revealed a main effect for housing ($F [3, 60] = 8.634$, $p < 0.001$), a main effect for time ($F [1, 60] = 145.377$, $p < 0.001$), and an interaction between the two ($F [3, 60] = 8.578$, $p < 0.001$). In terms of the main effect for housing, *Post hoc* analyses revealed that non-enriched, physically-enriched, and socially-enriched animals all engaged in greater amounts of voluntary exercise than did super-enriched animals during the first minute of activity (NE=PE=SE>Sup). Specifically for the main effect for time, animals engaged in

less voluntary activity during the first minute of activity during the second measurement than during the first measurement. In terms of the interaction, the pattern of number of housing differences changed from week two to week four. In week two, the physically-enriched animals engaged in more exercise during the first minute of activity than did the non-enriched and socially-enriched, who engaged in more exercise during the first minute of activity than did the super-enriched (PE>NE=SE>Sup). This pattern reflected an inverse U-shaped curve for enrichment, such that some enrichment (i.e., the physically enriched group) resulted in the greatest level of activity, whereas no enrichment (i.e., the non-enriched group) and too much enrichment (i.e., the socially- and super-enriched groups) resulted in less activity. In week four, the pattern changed to a linear, negative correlation between enrichment and exercise, so that as the amount of enrichment increased, the amount of exercise engaged in decreased (NE>PE>SE>Sup).

Discussion for Experiment I

The purpose of this experiment was to examine the effects of environmental enrichment on body weight, food consumption, and activity. Previous experiments have found that enrichment attenuates body weight gain, has minimal effects on food consumption, and has differing effects on activity. The effects on activity depend on the type of activity measured. Specifically, enrichment decreases open field activity and increases home cage activity. The decrease in open field activity is attributed to habituation in a novel environment. The increase in home cage activity has not yet been explained. Greater amounts

of enrichment (i.e., physical and social) have been demonstrated to have greater effects on body weight and activity. Environmental enrichment, therefore, appears to work in a "dose-response" manner, so that greater amounts of enrichment have greater effects. This experiment was designed to replicate previous work, as well as to extend it by examining environmental enrichment's effects on an additional measure of activity: voluntary exercise.

The present experiment examined the dependent variables of body weight, food consumption, open field activity, home cage activity, and voluntary exercise to determine the effects of various housing conditions. The findings of Tomchesson (2006) and Shafer (2006) were partially replicated in the present study.

Several similarities exist between the current experiment and previous experiments on this topic – specifically, environmental enrichment's effects on body weight, food consumption, and open field activity. Environmental enrichment resulted in attenuated body weight gain over time. This finding is consistent with both Tomchesson (2006) and Shafer (2006), indicating that environmental enrichment's effects on body weight are consistent. Social enrichment initially resulted in decreased food consumption, but this effect reversed by the end of the experiment, such that socially-enriched animals had greater food consumption than non-socially-enriched animals. The initial decrease in food consumption for the socially-enriched animals is consistent with Shafer (2006), but the reversal is not. This inconsistency may reflect differences in design. Specifically, Shafer (2006) measured environmental enrichment's

effects on food consumption for only three weeks whereas the current project measured environmental enrichment's effects on food consumption for five weeks. It is possible that if Shafer (2006) had measured food consumption for the same period of time, then she also may have found a reversal in social enrichment's effects on food consumption. Enrichment decreased open field activity, which is also consistent with past research (Shafer, 2006; Tomchesson, 2006).

Several key differences exist between the current experiment and previous experiments on this topic, specifically environmental enrichment's effects on home cage activity and voluntary exercise. Environmental enrichment decreased home cage activity, which was not consistent with previous findings. This difference could be the result of a variety of factors. This experiment differed from Tomchesson (2006) in that the super-enriched cages housed eight animals each rather than twelve animals each. Although the present experiment was based on Tomchesson (2006), procedural differences may contributed to the difference in finding. Time of day, and diurnal variations in activity, could have contributed if home cage activity was measured at different times.

Environmental enrichment had differential effects on exercise, depending on type and amount of enrichment. Physical enrichment increased exercise, whereas combined physical and social enrichment led to decreased voluntary exercise. Social enrichment alone did not seem to influence voluntary exercise, as it did not differ from non-enrichment in terms of amount of voluntary exercise. It seems that type of enrichment and amount of enrichment are critical factors in

determining voluntary activity, specifically exercise. Physical enrichment may have led to familiarity with physical objects, such that the rats in the physical enrichment condition may have felt more comfortable interacting with physical objects in a novel environment. Habit also may be the mechanism by which physical enrichment leads to increased voluntary exercise. If individuals habitually interact with physical objects, then they may be more likely to do so in a novel situation. Social enrichment may not have any impact on physical activity in a novel environment, but may affect physical activity in a familiar environment. Combined physical and social enrichment may have led to decreased voluntary exercise for several reasons. Similarly to open field, greater enrichment in the home cage could have led to habituation in a novel environment because the novel environment was not as stimulating as the home cage. The lack of stimulation may have led to either boredom or relaxation. It is also possible that the interaction of physical and social enrichment somehow leads to decreased voluntary activity in a novel environment. These findings may have implications for altering the level of physical activity within a sedentary population. If physical enrichment increases voluntary exercise through familiarity, then increasing familiarity with different types of exercise and exercise equipment may lead to increased exercise. If physical enrichment increases voluntary exercise through habit, then strengthening the habit in individuals may also lead to increased exercise. Additionally, if combined enrichment decreases voluntary exercise, creating either (a) home environments that are less stimulating than a novel environment or (b) novel environments that are as or

more stimulating than a home environment, also may lead to increased exercise. In humans, if an individual's exercise environment, such as a gym or health club, is more stimulating than an individual's home, then the individual may be more likely to exercise than if the reverse were true.

Overall, these findings also indicate that food consumption and activity differences cannot account for body weight differences amongst differentially enriched conditions. Environmental enrichment, therefore, may have direct effects on body weight. These direct effects may work through increasing basal metabolic rate, or increasing mircromovements.

Experiment IIa

Overview

This experiment was designed to evaluate effects of nicotine on physical activity, food consumption, and body weight of rats raised and living in different housing conditions. There were two different drug conditions (nicotine or saline). There were four different housing conditions that manipulated the social and physical environment. Physical activity was measured in two different ways: movement in an open field locomotor chamber, and activity in exercise wheels. This experiment included a three-week drug administration phase. The experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC) and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH, 1996).

Hypotheses

1. Enriched housing will:
    a. decrease body weight, with super-enrichment having the greatest effects. This hypothesis is based on the findings of Shafer (2006) and Tomchesson (2006): both found that super-enrichment attenuates body weight gain.
    b. have minimal effects on food consumption. This hypothesis is based on the findings of Shafer (2006) and Tomchesson (2006): both found that enrichment had minimal effects on food consumption.
    c. decrease open field activity, with greater enrichment having greater effects (NE<PE=SE<Sup). This hypothesis is based on the findings of Shafer

(2006) and Tomchesson (2006): both found that enrichment, especially super-enrichment, decreased open field activity.

2. Nicotine will:
    a. decrease body weight. This hypothesis is based on many findings in our laboratory that nicotine decreases body weight (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg, 1992; Winders & Grunberg, 1989).
    b. decrease food consumption. This hypothesis is based on many findings in our laboratory that nicotine decreases food consumption (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg, 1992; Winders & Grunberg, 1989).
    c. increase open field activity. This hypothesis is based on many findings in our laboratory that nicotine increases open field activity (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg & Bowen, 1985).
    d. increase voluntary exercise. This hypothesis is based on many findings in our laboratory that nicotine increases open field activity (e.g., Faraday, Elliott, & Grunberg, 2001; Grunberg & Bowen, 1985). It was hypothesized that these effects on activity would generalize across all types of activity, including voluntary exercise.

No hypothesis was made for the effects of enriched housing on voluntary exercise because of the lack of discussion in the literature. In addition, no hypotheses were made for the effects of enriched housing on nicotine's effects on body weight, food consumption, and activity. Literature on this topic was lacking, and it was unclear if enriched housing would attenuate, potentiate, add to, or nullify nicotine's effects on these behavioral variables.

Subjects

Subjects were the same 64 male Sprague Dawley rats (Charles River Laboratories) from Experiment I. The subjects were 56 days old at the beginning of this experiment and weighed between 229.15 and 370.32 grams, with a mean of 317.32 grams.

Housing

Subjects were maintained in the same housing conditions that they were assigned to for Experiment I. They remained in these housing conditions throughout Experiment IIa.

Procedures

Half of the subjects from each housing condition received saline and half received nicotine. Animals from the NE and PE conditions were assigned to drug condition such that the body weights of the groups were comparable at the beginning of Experiment IIa. Animals from the SE and SUPER conditions were assigned to drug conditions by cage and an attempt was made to match the body weights by cage. Drug was administered for 18 days via subcutaneously-implanted osmotic minipumps. Throughout the experiment, food consumption (FC) was measured every other day. Open field (OF) was measured twice during the drug administration phase (two weeks apart). Activity in exercise wheels (EX) was measured once during the drug administration phase (during the week when OF was not measured). Body weight (BW) was measured weekly before either the OF or EX measure. Due to logistical considerations, not all animals' open field activity and voluntary exercise were measured on the

same day. Animals were split into two cohorts for open field activity and four cohorts for voluntary exercise an equal number of animals from each housing condition were evaluated during each measurement. As body weights were measured before OF or EX, body weights for the different cohorts were measured on different days. These differences are reflected in the timeline below, such that BW (all) indicates that all animals' body weights were measured on a given day, BW (1/2) and OF (1/2) indicate that half of the animals' body weights and open field activity were measured on a given day, and BW (1/4) and EX (1/4) indicate that a quarter of the animals' body weights and voluntary exercise were measured on a given day.

Experiment IIa Timeline	
Day	**Measures Taken**
38	Implant (1/2)
39	FC, Implant (1/2)
40	BW (1/2), OF (1/2)
41	BW (1/2), OF (1/2), FC
42	
43	BW (1/4), Ex (1/4), FC, Change T+C
44	BW (1/4), Ex (1/4)
45	BW (1/4), Ex (1/4), FC
46	BW (1/4), Ex (1/4), Change T+C
47	FC
48	
49	FC
50	BW (1/2), OF (1/2), Change T+C
51	FC
52	
53	BW (1/2), OF (1/2), FC, Change T+C
54	
55	FC

Body weight and food consumption. Body weight (BW) and food consumption (FC) measurements followed the same procedures as described under Experiment I (above).

Activity measurements: OF, EX. Activity measurements followed the same procedures as described under Experiment I (above) except for the number of measurements taken.

Drug administration. After Experiment I (which also served as a pre-drug phase for Experiment II), osmotic mini-pumps containing saline or 9 mg/kg/day of nicotine dihydrochloride were surgically implanted into subjects. The form and dosage of nicotine was based on previous reports (Grunberg & Bowen, 1985; Winders & Grunberg, 1990; Faraday, Scheufele, Rahman, & Grunberg, 1999; Scheufele, Faraday, & Grunberg, 2000; Elliott et al., 2004). Nicotine dihydrochloride was dissolved in physiologic saline (0.9% NaCl) and placed in Alzet osmotic minipumps (Model 2002, Durect Corporation). Surgeries were conducted in a separate Laboratory Animal Management (LAM) procedure room equipped with anesthesia equipment and an operating table. The animals were anesthetized with isoflurane mixed with oxygen using a vaporizer with flowmeter. The percentage of isoflurane-oxygen mix was determined based on recommendations from LAM personnel. The animals were placed inside a Plexiglas induction chamber saturated with the anesthesia. After tail-pinch produced no reflexive movement (after approximately 2 minutes), the animals were removed from the induction chamber, placed on an absorbent surgical pad, and fitted with a nose cone attached to the vaporizer to prevent pain by delivering

constant anesthesia through the entire surgical procedure. A 2 x 2.5 em area on each animal's back was shaved with electric clippers. After swabbing with a Betadine antiseptic solution, a small cut-approximately 1 centimeter in length was made through the skin between the withers (shoulder blades) of each animal with surgical scissors, and the pumps were inserted beneath the skin, with the pump opening toward the animal's posterior. The incisions were closed with stainless steel 9 millimeter wound clips. Each animal was monitored until fully awake, alert, and able to ambulate. The order of the surgical procedures was counterbalanced in order to alternate nicotine and saline minipump implantation.

Data Analytic Strategy for Experiment IIa

Subjects maintained assignment to housing conditions from Experiment I. Subjects were assigned to drug condition as described above in the Procedures section. Although analyses of variance (ANOVA) were used for all data analyses, the particular version of ANOVA varied based on the dependent variable under study.

Body weight and food consumption were analyzed using repeated-measures ANCOVAs to assess over time throughout the experiment. The last body weight and food consumption measurements from the previous experiment were used as covariates. Any significant main effects or interactions were examined using separate ANOVAs following the procedures of Keppel (1991). If there was a significant effect, then Tukey HSD *post-hoc* analyses were performed. F values, degrees of freedom and p values for analyses in Experiment IIa are provided in Appendix B.

Open-field activity was analyzed using repeated-measures ANOVAs to examine the effects of enrichment on locomotor activity. For all open-field activity analyses, enrichment and drug condition were the between-subjects factors and time was the within-subjects factor. Three separate repeated-measures ANOVAs were computed for each of three different types of activity recorded in the open-field chambers (i.e., horizontal activity, vertical activity, and center time). Although all three measures are conceptually related and could be analyzed with a multivariate ANOVA, the changes in these types of activity over time were of greater interest than how the three were related within a single open-field activity session. In addition, center time ratios were computed and analyzed with a repeated-measures ANOVA. Within-session open-field activity was also analyzed using a repeated measure ANOVA. If the initial analyses indicated significant between-subjects effects, then repeated-measures ANOVA were performed separately for each of the open-field trials.

Exercise was analyzed with a two-way ANOVA as only one exercise measurement was taken. Latency to start movement in the activity wheel was analyzed similarly. The number of full wheel rotations during the first minute of activity was also analyzed using an ANOVA. Similarly to open-field activity, within-session exercise was analyzed using a repeated-measures ANOVA. Enrichment and drug condition were the between-subject factors and time was the within-subject factor. If the initial analyses indicated significant between-subjects effects, then repeated-measures ANOVA were performed separately for each of the open-field trials.

Several strategies were used to minimize the probability of Type I and Type II error. First, only if overall analyses revealed a significant main effect or interaction were subsequent analyses performed. This strategy reduces the number of statistical tests performed (Keppel, 1991; Cohen & Cohen, 1983). All tests were two-tailed with significance determined by $p \leq 0.05$. In addition, the experiment was designed to provide adequate power (0.80). Type II error is minimized when sample size supports adequate power (Keppel, 1991).

Data were excluded from the analyses only if two criteria were met: (1) data points were more than three standard deviations from the mean of the experimental condition corresponding to those data, and (2) data were inconsistent with the subject's scores over time. To determine inconsistency, each datum was compared with the subject's previous and next datum for that particular subject. If disparate, the data were excluded from analyses. Three data points of 960 total data points were excluded from analyses, all of which came from the food consumption data set. Specifically for food consumption, if a datum was two times greater or less than the previous and next datum, or was a negative change score (i.e., the food weight had increased from one measurement to the next), it was considered inconsistent. If the datum was also three standard deviations from the mean, then it was excluded from data analyses.

Results for Experiment IIa

Body weight (see Figure 8). The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. Repeated-measures ANOVA with previous body weight as a covariate revealed a main effect for housing ($F [3, 55] = 3.227$, $p < 0.05$) and a main effect for drug condition ($F [1, 55] = 129.669$, $p < 0.001$), but no main effect for time. Specifically, non-enriched animals had greater body weights than did animals from the other housing groups. Animals in the saline condition had greater body weights than did animals in the nicotine condition. A time by drug interaction was present ($F [1.177, 64.722] = 16.701$, $p < 0.001$), indicating that animals in the nicotine condition exhibited attenuated weight gain as compared with animals in the saline condition.

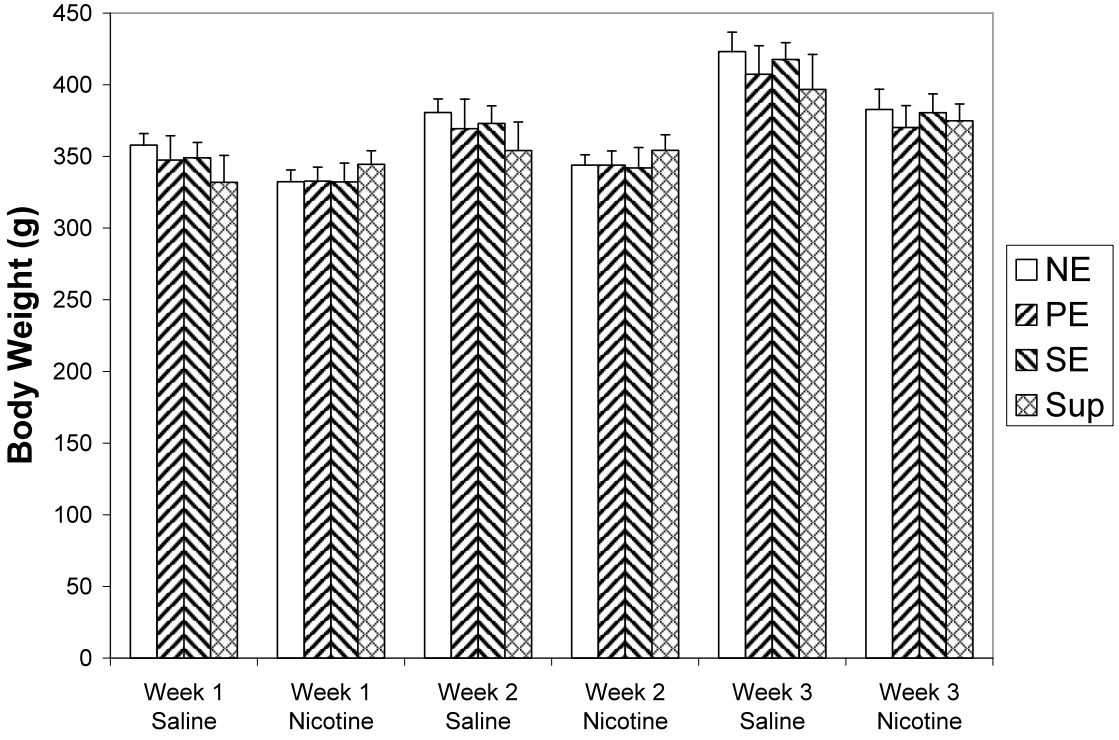

Figure 8. Mean body weight (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

Food consumption (see Figure 9). The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. A repeated-measures ANOVA with previous food consumption as a covariate revealed a main effect for drug ($F [1, 54] = 73.150$, $p < 0.001$), but no main effect for housing or time. Several interactions were evident: a time by housing interaction ($F [5.233, 94.187] = 2.529$, $p < 0.05$),

a time by drug interaction ($F [1.744, 94.187] = 37.822$, $p < 0.001$), and a time by housing by drug interaction ($F [5.233, 94.187] = 2.371$, $p < 0.05$).

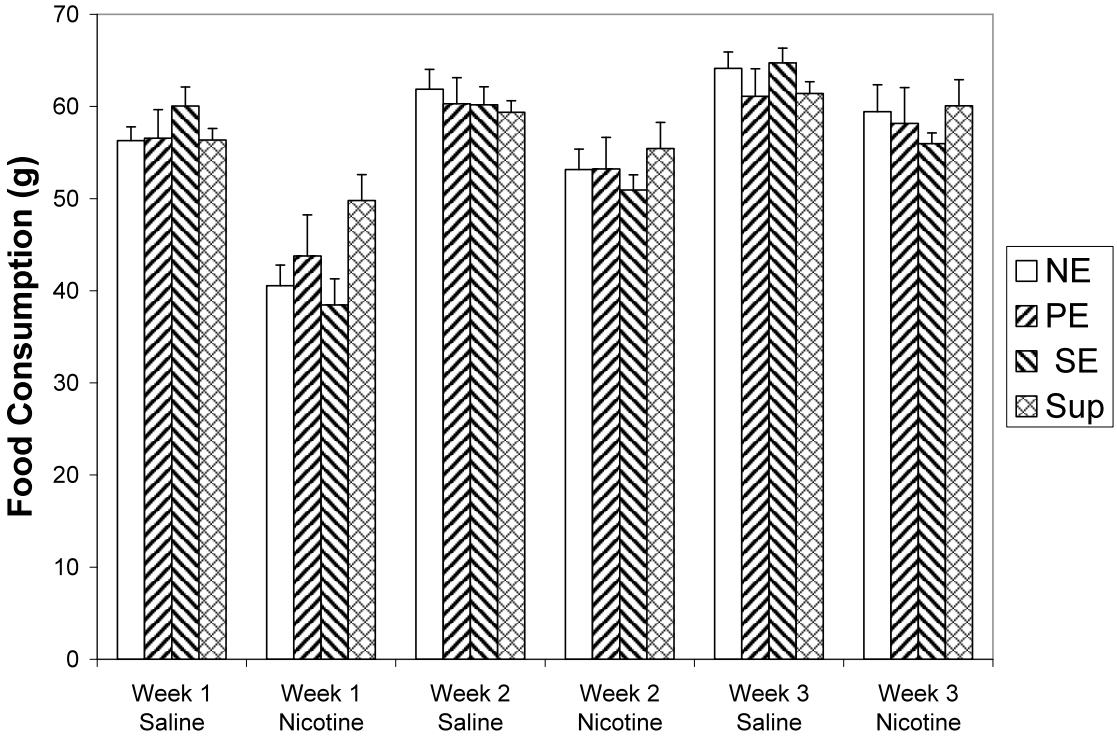

Figure 9. Mean food consumption (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

Open field activity (see Figures 10-12). The repeated-measures ANOVA for horizontal activity revealed a main effect for housing ($F [3, 56] = 32.142$, $p < 0.001$), but no main effects for time or drug and no interactions. *Post hoc* analyses revealed that non-enriched animals engaged in greater amounts of horizontal activity than did physically- and socially-enriched animals, who

engaged in greater amounts of horizontal activity than did super-enriched animals (NE>PE=SE>Sup).

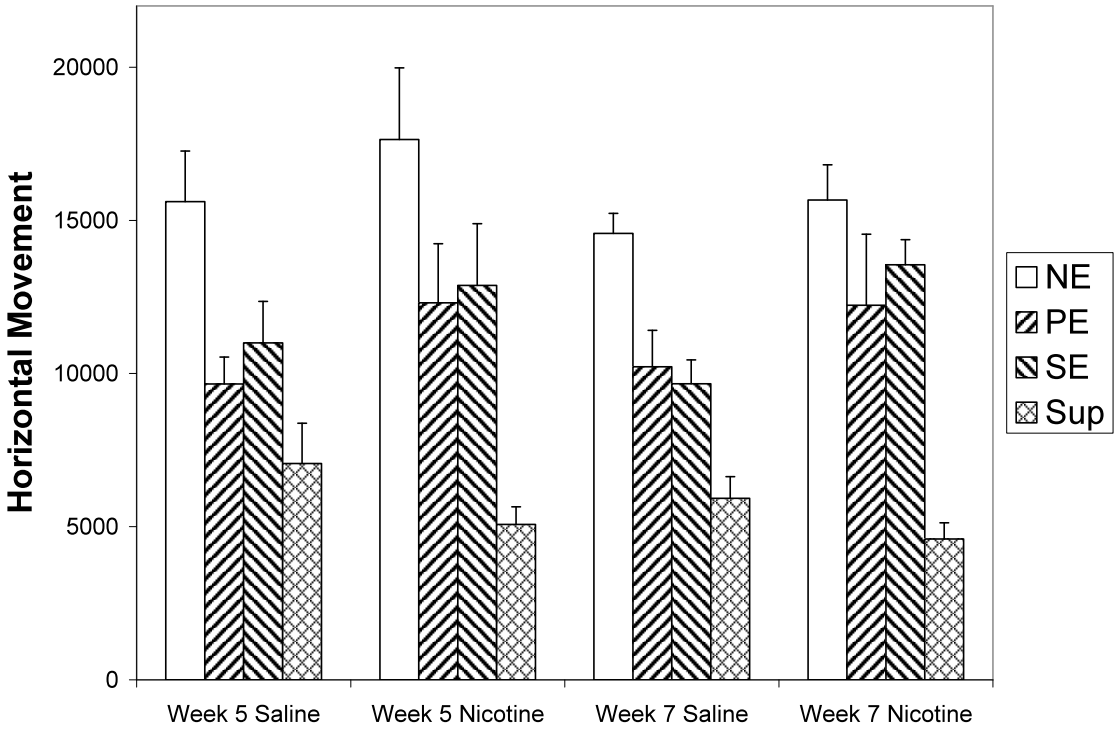

Figure 10. Mean open field horizontal activity (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The repeated-measures ANOVA for vertical activity revealed a main effect for time ($F [1, 56] = 12.991$, $p < 0.001$), a main effect for housing ($F [3, 56] = 17.837$, $p < 0.001$), a main effect for drug ($F [1, 56] = 8.738$, $p < 0.01$), and a housing by drug interaction ($F [3, 56] = 3.429$, $p < 0.05$). The overall amount of

vertical activity increased from the first open field measurement to the second open field measurement. *Post hoc* analyses revealed that non-enriched animals had greater amounts of vertical activity than did all other housing conditions (NE>PE, SE, Sup). In addition, socially-enriched animals had greater vertical activity than did super-enriched animals (SE>Sup). Physically-enriched animals did not differ significantly from socially- and super-enriched animals. Animals in the saline condition had greater amounts of vertical activity than did animals in the nicotine condition. Animals in the nicotine, socially-enriched group had greater amounts of vertical activity than did animals in the saline, socially-enriched group (SE: Nic>Sal), whereas the reverse pattern was true in the other three housing conditions (NE, PE, Sup: Nic<Sal).

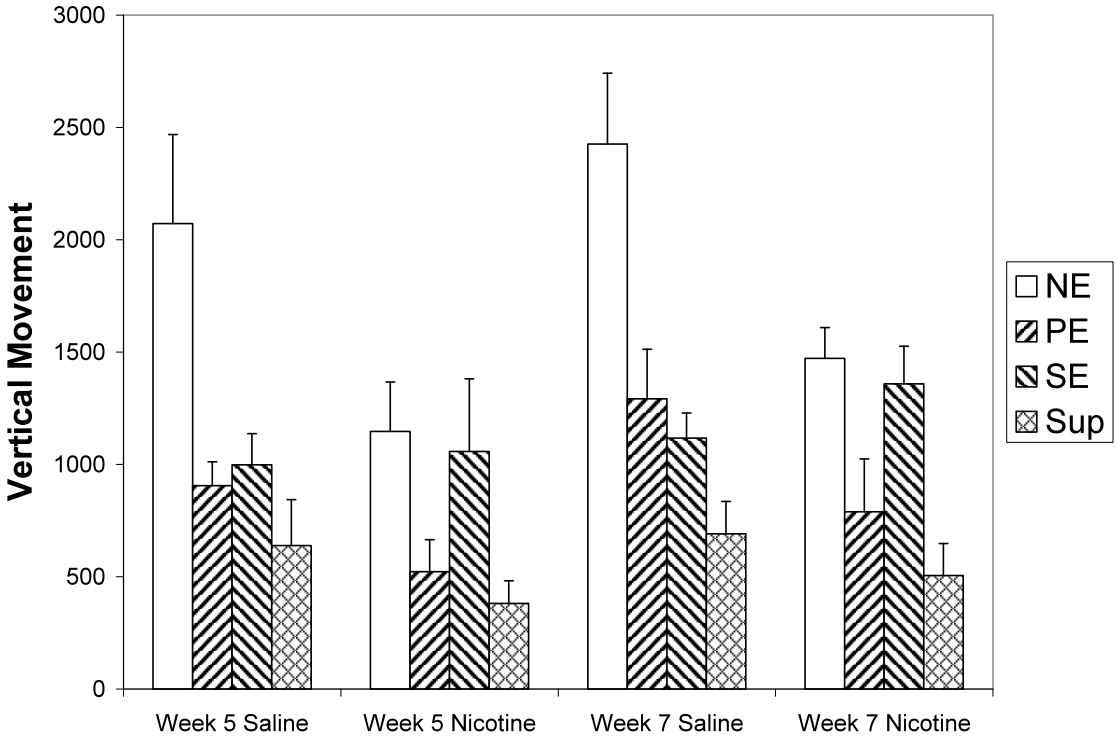

Figure 11. Mean open field vertical activity (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The repeated-measures ANOVA for center time revealed a main effect for housing ($F [3, 56] = 3.329$, $p < 0.05$) and a time by housing interaction ($F [3, 56] = 5.227$, $p < 0.01$). *Post hoc* analyses revealed that non-enriched animals had greater amounts of center time activity than did super-enriched animals (NE>Sup). Non-enriched animals maintained their center time activity from the first open field measurement to the second open field measurement, whereas both the physically- and socially-enriched animals increased their center time

activity from the first to the second open field measurement. In contrast, the super-enriched animals decreased their center time activity from the first to the second open field measurement.

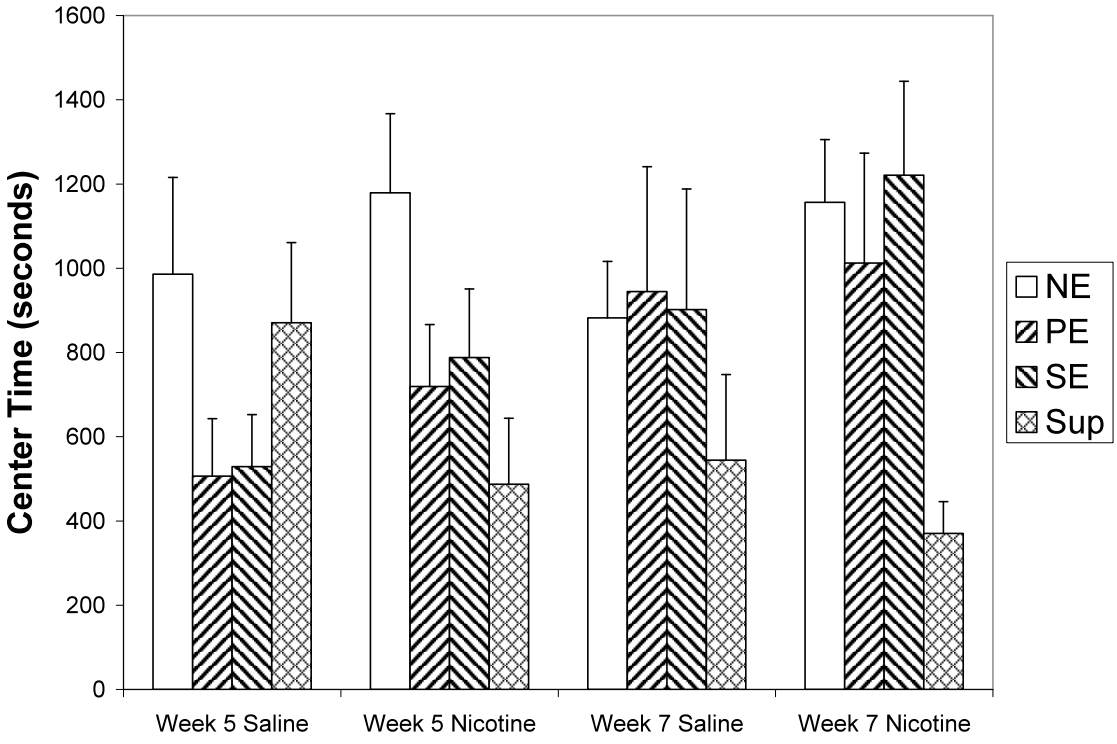

Figure 12. Mean open field center time activity (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The repeated-measures ANOVA for the first within-session open field activity also

yielded statistically significant results. The analysis revealed a main effect for time ($F [5.839, 321.152] = 171.765$, $p < 0.001$), a main effect for housing condition ($F [3, 55] = 16.317$, $p < 0.001$), and an interaction between the two ($F [17.517, 321.152] = 3.632$, $p < 0.001$). *Post-hoc* analyses revealed that the non-enriched condition had greater amounts of activity than all other conditions. The physically-enriched and socially-enriched conditions also had greater amounts of activity than the super-enriched condition.

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The repeated-measures ANOVA for the second within-session open field activity also yielded statistically significant results. The analysis revealed a main effect for time ($F [7.020, 393.099] = 193.369$, $p < 0.001$) and a main effect for housing condition ($F [3, 56] = 28.782$, $p < 0.001$]. The analysis also revealed a time by housing interaction ($F [21.059, 393.099] = 2.369$, $p < 0.001$). *Post-hoc* analyses revealed that the non-enriched condition had greater amounts of activity than all other conditions. The physically-enriched and socially-enriched conditions also had greater amounts of activity than the super-enriched condition.

Exercise (see Figure 13). A two-way ANOVA revealed a main effect for drug ($F [1, 56] = 7.249$, $p < 0.01$), but no housing by drug interaction. *Post hoc* analyses for the drug effect revealed that animals given nicotine engaged in greater amounts of exercise than animals given saline.

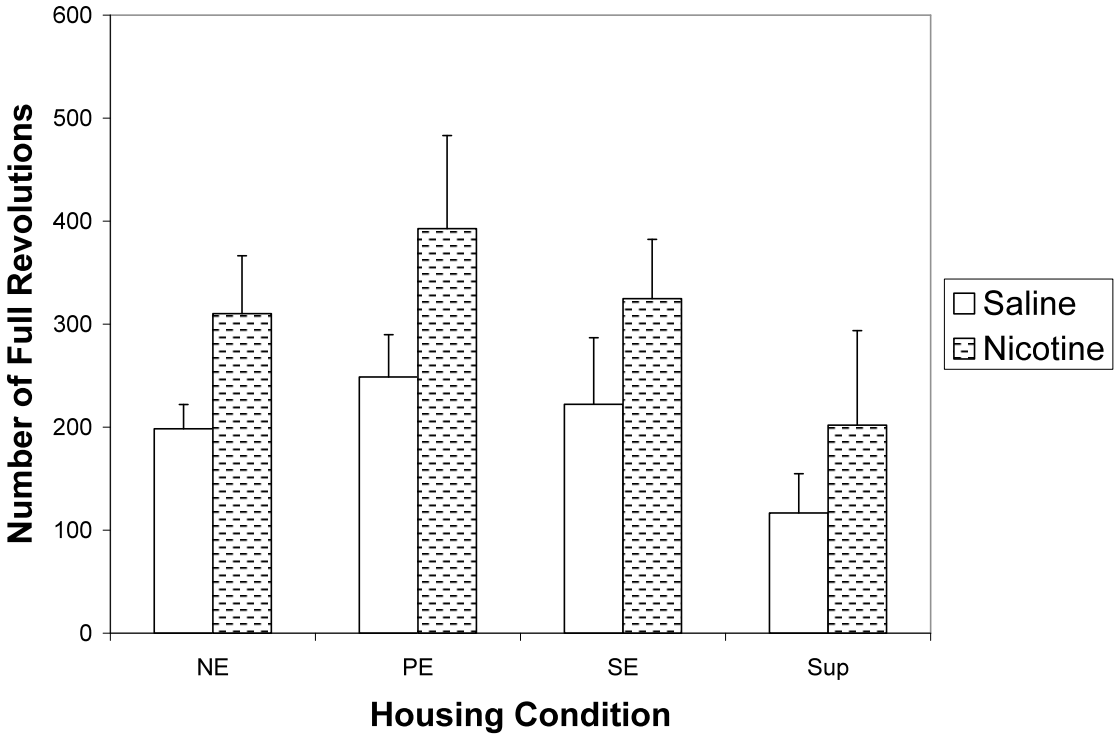

Figure 13. Mean voluntary exercise (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. Within-session repeated-measures ANOVA revealed a main effect for drug ($F [1, 56] = 7.260$, $p < 0.01$) and a main effect for time ($F [1.160, 64.978] = 156.960$, $p < 0.001$). *Post hoc* analyses revealed that physically-enriched animals engaged in greater amounts of voluntary exercise than super-enriched animals. Animals given nicotine engaged in greater amounts of voluntary exercise than animals

given saline. The within-session repeated-measures ANOVA also revealed two interactions: a time by housing interaction ($F [3.481, 64.978] = 2.851$, $p < 0.05$) and a time by drug interaction ($F [1.160, 64.978] = 6.543$, $p < 0.01$). For the time by housing interaction, the differences between housing conditions changed over time. Initially, the physically-enriched animals engaged in more exercise than did the non-enriched and socially-enriched animals, both of which groups engaged in more exercise than did the super-enriched animals (PE>NE=SE>Sup). Over time, the non-enriched, physically-enriched, and socially-enriched animals all engaged in more exercise than did the super-enriched animals (NE=PE=SE>Sup).

A two-way ANOVA for latency to start indicated a housing by drug interaction ($F [3, 56] = 4.968$, $p < 0.01$), but no main effects for housing or for drug. Specifically, non-enriched and physically-enriched animals had less latency to start than super-enriched animals.

A repeated-measures ANOVA for number of full revolutions during the first minute of activity revealed no statistically significant differences between housing or drug conditions.

Discussion for Experiment IIa

The purpose of this experiment was to examine the direct effects of environmental enrichment and nicotine on body weight, food consumption, and activity, as well as the effects of environmental enrichment on nicotine's effects on the same dependent variables. Although previous experiments have examined environmental enrichment's effects on body weight, food consumption,

and activity as well as chronic nicotine's effects on body weight food, consumption, and activity, no one has examined how environmental enrichment may alter chronic nicotine's effects on these dependent variables. It is unclear as to whether they may have additive, competitive, or interactive effects. Previous experiments have examined how environmental enrichment may alter acute nicotine's effects on these dependent variables. The present experiment examined the dependent variables of body weight, food consumption, open field activity, and voluntary exercise to determine how environmental enrichment may alter nicotine's effects.

Environmental enrichment attenuated body weight gain and nicotine also attenuated body weight gain. Environmental enrichment does not appear to have altered nicotine's effects, suggesting that they work through different mechanisms to attenuate weight gain. Unlike in Experiment I, the rates of body weight gain did not differ over time for the different housing conditions. It is possible that the significant decrease in the animals' rate of growth (e.g., they were adults during Experiment IIa but adolescents during Experiment I) during Experiment IIa could account for this difference.

Environmental enrichment had no effect on food consumption, whereas nicotine decreased food consumption. It appears that environmental enrichment may potentiate nicotine's effects on food consumption for physical and social enrichment, but not for super-enrichment.

Environmental enrichment decreased open field activity, which is consistent with previous experiments. Nicotine decreased only vertical activity in

the open field chamber. There was a housing by drug interaction in the second measurement of vertical activity. Nicotine, however, did not seem to alter within-session horizontal activity.

Environmental enrichment had differential effects on exercise, depending on type and amount of enrichment. Physical enrichment increased exercise, whereas combined physical and social enrichment led to decreased voluntary exercise. Social enrichment alone did not seem to influence voluntary exercise, as it did not differ from non-enrichment in terms of amount of voluntary exercise. Nicotine increased exercise, but there was no apparent interaction between housing and drug because nicotine similarly increased activity across all environmental conditions.

Overall, these findings indicate that environmental enrichment's effects on body weight, food consumption, and activity cannot fully account for body weight differences. Nicotine's effects on body weight may be accounted for by decreased food consumption or increased exercise. Environmental enrichment and nicotine had few interactions. It appears that environmental enrichment alters nicotine's actions on food consumption.

Experiment IIb

Overview

This experiment was designed to evaluate effects of nicotine cessation on physical activity, food consumption, and body weight of rats raised and living in different housing conditions. There were two different drug conditions (nicotine or saline). There were four different housing conditions that manipulated the social and physical environment. Physical activity was measured in two different ways: movement in an open field locomotor chamber, and activity in exercise wheels. This experiment included a three-week post-drug phase. The experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC) and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH, 1996).

Hypotheses

1. Enriched housing will:
    a. decrease body weight, with super-enrichment having the greatest effects. This hypothesis is based on the findings of Shafer (2006) and Tomchesson (2006): both found that super-enrichment attenuates body weight gain.
    b. have minimal effects on food consumption. This hypothesis is based on the findings of Shafer (2006) and Tomchesson (2006): both found that enrichment had minimal effects on food consumption.
    c. decrease open field activity, with greater enrichment having greater effects (NE<PE=SE<Sup). This hypothesis is based on the findings of Shafer

(2006) and Tomchesson (2006): both found that enrichment, especially super-enrichment, decreased open field activity.

2. Nicotine cessation will:
    a. increase body weight. This hypothesis is based on the findings of Grunberg, Bowen, and Winders (1986) and Perry (2007), who found that nicotine cessation resulted in increased body weight.
    b. increase food consumption. This hypothesis is based on the findings of Winders and Grunberg (1989), who found that nicotine cessation resulted in increased food consumption.
    c. decrease open field activity. This hypothesis is based on the findings of Perry (2007), who found that nicotine cessation resulted in decreased open field activity for Sprague Dawley rats.
    d. decrease voluntary exercise. This hypothesis is based on the findings of Perry (2007), who found that nicotine cessation resulted in decreased open field activity for Sprague Dawley rats. It was hypothesized that these findings would generalize to all forms of activity, including voluntary exercise.

No hypothesis was made for the effects of enriched housing on voluntary exercise because of the lack of discussion in the literature. In addition, no hypotheses were made for the effects of enriched housing on nicotine cessation's effects on body weight, food consumption, and activity. Literature on this topic was lacking, and it was unclear if enriched housing would attenuate,

potentiate, add to, or nullify nicotine cessation's effects on these behavioral variables.

Subjects

Subjects were the same 64 male Sprague Dawley rats (Charles River Laboratories) from Experiment IIa. The subjects were 74 days old at the beginning of this experiment and weighed between 305.8 and 576 grams, with a mean of 430.31 grams.

Housing

Subjects were maintained in the same housing conditions that they were assigned to for Experiment IIa. They remained in these housing conditions throughout Experiment IIb.

Procedures

Animals were assigned to drug condition during Experiment IIa. These conditions were maintained through Experiment IIb. Osmotic minipumps were removed to begin the drug cessation phase. Throughout the experiment, food consumption (FC) was measured every other day. Open field (OF) was measured twice during the drug cessation phase (two weeks apart). Activity in exercise wheels (EX) was measured once during the drug cessation phase (during the week when OF was not measured). Body weight (BW) was measured weekly before either the OF or EX measure. Due to logistical considerations, not all animals' open field activity and voluntary exercise were measured on the same day. Animals were split into two cohorts for open field activity and four cohorts for voluntary exercise an equal number of animals from

each housing condition were evaluated during each measurement. As body weights were measured before OF or EX, body weights for the different cohorts were measured on different days. These differences are reflected in the timeline below, such that BW (all) indicates that all animals' body weights were measured on a given day, BW (1/2) and OF (1/2) indicate that half of the animals' body weights and open field activity were measured on a given day, and BW (1/4) and EX (1/4) indicate that a quarter of the animals' body weights and voluntary exercise were measured on a given day.

Experiment IIb Timeline	
Day	**Measures Taken**
56	Explant (1/2)
57	FC, HCA, Explant (1/2)
58	
59	BW (1/2), OF (1/2), FC
60	BW (1/2), OF (1/2), Change T+C
61	FC
62	
63	FC
64	BW (1/4), Ex (1/4), Change T+C
65	BW (1/4), Ex (1/4), FC
66	BW (1/4), Ex (1/4)
67	BW (1/4), Ex (1/4), FC, Change T+C
68	
69	FC
70	
71	BW (1/2), OF (1/2), FC
72	
73	FC
74	BW (1/2), OF (1/2)
75	FC
76	
77	FC, BW (all)
78	
79	FC, BW (all)
80	
81	FC, Euthanasia

Body weight and food consumption. Body weight (BW) and food consumption (FC) measurements followed the same procedures as described under Experiment I (above).

Activity measurements: OF, EX. Activity measurements followed the same procedures as described under Experiment I (above) except for the number of measurements taken.

Drug withdrawal. After 18 days of saline or nicotine administration, the osmotic minipumps were surgically explanted and the post-drug phase began. Explant followed similar procedures as implant (described in Experiment IIa Methods), except that the minipumps were removed rather than inserted.

Euthanasia. All animals were sacrificed by decapitation 3 ½ weeks after the explant surgery. Blood, brain, and tissue samples were collected for further research.

Data Analytic Strategy for Experiment IIb

Subjects were maintained assignment to housing and drug conditions from Experiment IIa. Although analyses of variance (ANOVA) were used for all data analyses, the particular version of ANOVA varied based on the dependent variable under study.

Body weight and food consumption were analyzed using repeated-measures ANCOVAs to assess over time throughout the experiment. The last body weight and food consumption measurements from the previous experiment were used as covariates. Any significant main effects or interactions were examined using separate ANOVAs following the procedures of Keppel (1991). If

there was a significant effect, then Tukey HSD *post-hoc* analyses were performed. F values, degrees of freedom and p values for analyses in Experiment IIb are provided in Appendix C.

Open-field activity was analyzed using repeated-measures ANOVAs to examine the effects of enrichment on locomotor activity. For all open-field activity analyses, enrichment and drug condition were the between-subjects factors and time was the within-subjects factor. Three separate repeated-measures ANOVAs were computed for each of three different types of activity recorded in the open-field chambers (i.e., horizontal activity, vertical activity, and center time). Although all three measures are conceptually related and could be analyzed with a multivariate ANOVA, the changes in these types of activity over time were of greater interest than how the three were related within a single open-field activity session. In addition, center time ratios were computed and analyzed with a repeated-measures ANOVA. Within-session open-field activity was also analyzed using a repeated measure ANOVA. If the initial analyses indicated significant between-subjects effects, then repeated-measures ANOVA were performed separately for each of the open-field trials.

Exercise was analyzed with a two-way ANOVA as only one exercise measurement was taken. Latency to start movement in the activity wheel was analyzed similarly. The number of full wheel rotations during the first minute of activity was also analyzed using an ANOVA. Similarly to open-field activity, within-session exercise was analyzed using a repeated-measures ANOVA. Enrichment and drug condition were the between-subject factors and time was

the within-subject factor. If the initial analyses indicated significant between-subjects effects, then repeated-measures ANOVA were performed separately for each of the open-field trials.

Several strategies were used to minimize the probability of Type I and Type II error. First, only if overall analyses revealed a significant main effect or interaction were subsequent analyses performed. This strategy reduces the number of statistical tests performed (Keppel, 1991; Cohen & Cohen, 1983). All tests were two-tailed with significance determined by $p \leq 0.05$. In addition, the experiment was designed to provide adequate power (0.80). Type II error is minimized when sample size supports adequate power (Keppel, 1991).
Data were excluded from the analyses only if two criteria were met: (1) data points were more than three standard deviations from the mean of the experimental condition corresponding to those data, and (2) data were inconsistent with the subject's scores over time. To determine inconsistency, each datum was compared with the subject's previous and next datum for that particular subject. If disparate, the data were excluded from analyses. Sixteen data points of 1,344 total data points were excluded from analyses, all of which came from the food consumption data set. Specifically for food consumption, if a datum was two times greater or less than the previous and next datum, or was a negative change score (i.e., the food weight had increased from one measurement to the next), then it was considered inconsistent. If the datum was also three standard deviations from the mean, then it was excluded from data analyses.

Results for Experiment IIb

Body weight (see Figure 14). The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. Repeated-measures ANOVA with previous body weight as a covariate revealed a main effect for drug condition ($F [1, 55] = 19.534$, $p < 0.001$), but no main effect for housing or time. Specifically, the animals given saline weighed more than the animals given nicotine. Three interactions were also present: a time by covariate interaction ($F [1.649, 90.686] = 12.946$, $p < 0.001$), a time by drug interaction ($F [1.649, 90.686] = 14.817$, $p < 0.001$), and a housing by drug interaction ($F [3, 55] = 4.818$, $p < 0.005$).

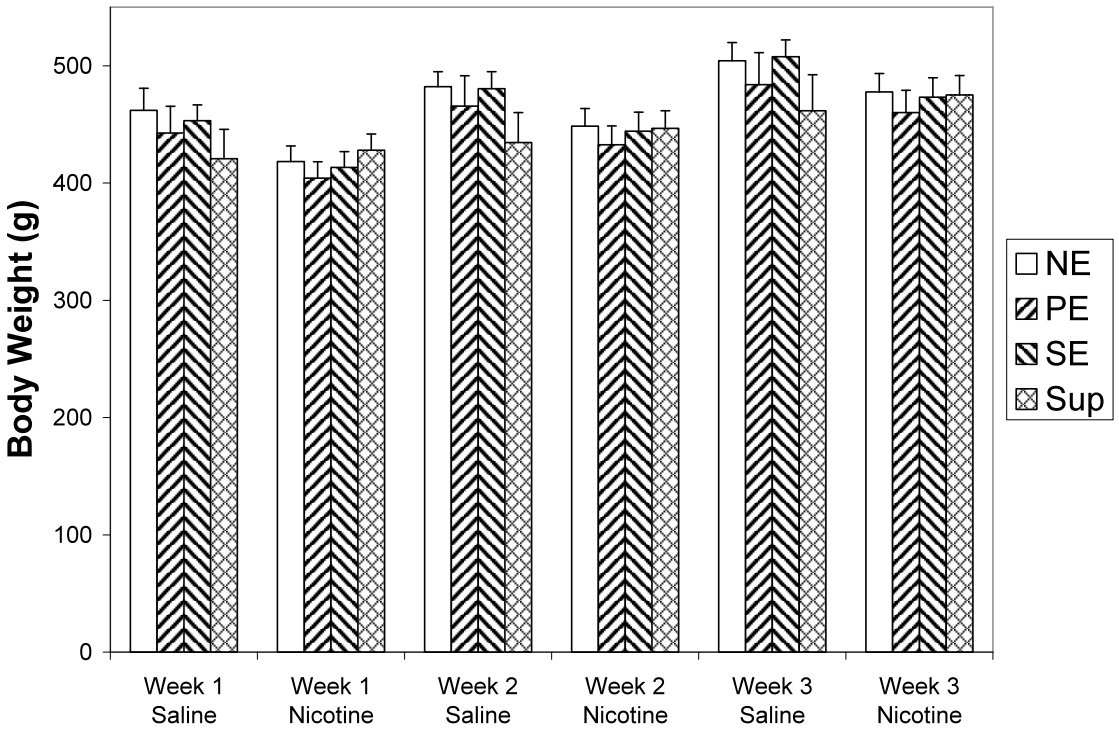

Figure 14. Mean body weight (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

Food consumption (see Figure 15). The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. A repeated-measures ANOVA revealed a main effect for time ($F [1.627, 86.299] = 13.134$, $p < 0.001$), but no main effect for housing or drug. Analyses did not reveal any interactions.

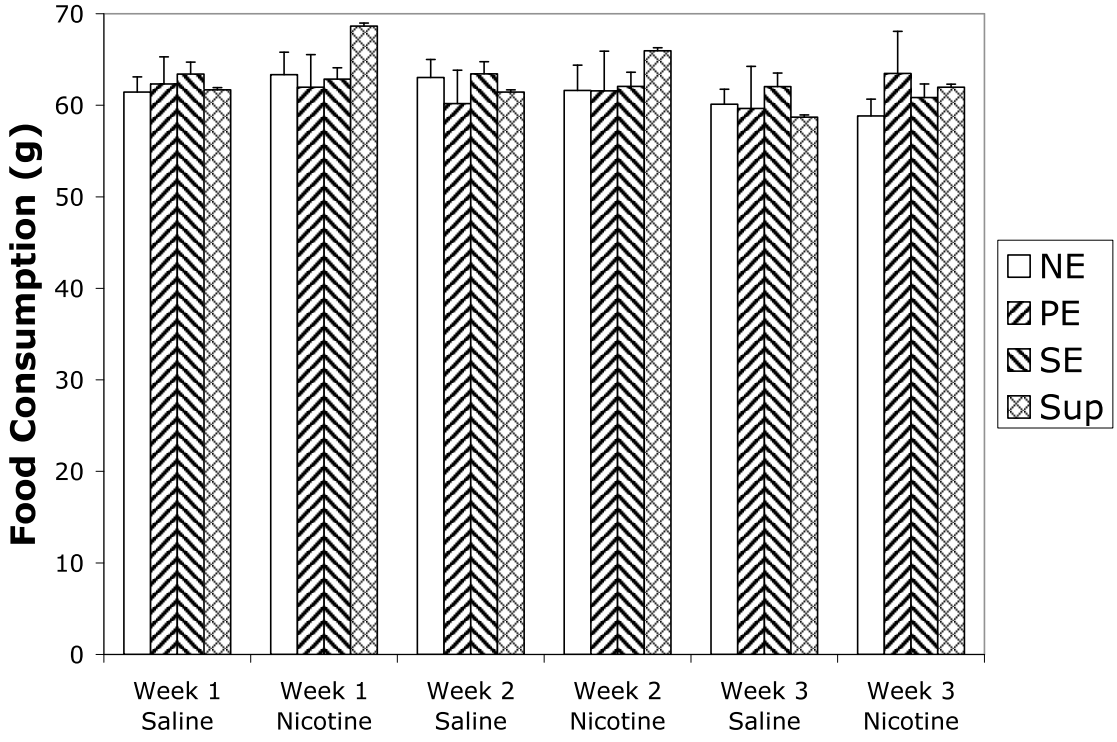

Figure 15. Mean food consumption (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

Open field activity (see Figure 16). The repeated-measures ANOVA for horizontal activity revealed a main effect for housing ($F [3, 56] = 24.877$, $p < 0.001$), but no other main effects and no interactions. *Post hoc* analyses revealed that the non-enriched, physically-enriched, and socially-enriched animals all had greater amounts of horizontal activity than did the super-enriched animals (NE=PE=SE>Sup).

The repeated-measures ANOVA for vertical activity revealed a main effect for housing ($F [3, 56] = 9.308$, $p < 0.001$), a time by housing interaction ($F [3, 56] = 6.193$, $p < 0.001$), and a housing by drug interaction ($F [3, 56] = 5.727$, $p < 0.01$). *Post hoc* analyses revealed that the non-enriched, physically-enriched, and socially-enriched animals all had greater amounts of vertical activity than did the super-enriched animals (NE=PE=SE>Sup). Animals in the non-enriched condition increased vertical activity greatly from the first open field measurement to the second open field measurement, whereas animals in both the socially-enriched condition and super-enriched condition maintained their vertical activity levels from the first to second open field measurement. In contrast, animals in the physically-enriched animals decreased vertical activity levels from the first to second open field measurement.

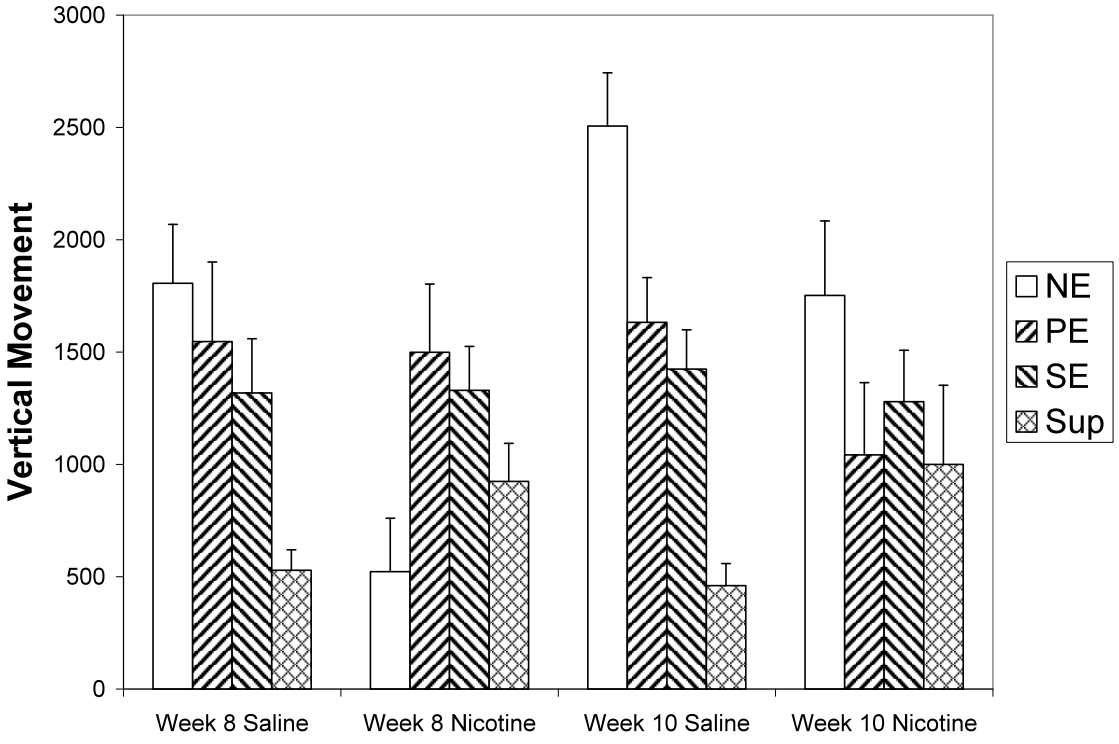

Figure 16. Mean open field vertical activity (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The repeated-measures ANOVA for center time revealed a main effect for housing ($F [3, 56] = 3.023$, $p < 0.05$), but no other main effects and no interactions. *Post hoc* analyses revealed that non-enriched animals had greater levels of center time activity than did super-enriched animals.

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The repeated-measures ANOVA for the first within-session open field activity also

yielded statistically significant results. The analysis revealed a main effect for time ($F [7.157, 400.817] = 267.726$, $p < 0.001$) and a main effect for housing condition ($F [3, 56] = 18.075$, $p < 0.001$), but no main effect for drug. The analysis also a time by housing interaction ($F [21.472, 400.817] = 6.016$, $p < 0.001$). *Post-hoc* analyses revealed that the non-enriched, physically-enriched, and socially-enriched conditions all had greater amounts of activity than the super-enriched condition.

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. The repeated-measures ANOVA for the second within-session open field activity also yielded statistically significant results. The analysis revealed a main effect for time ($F [7.392, 413.953] = 217.414$, $p < 0.001$), a main effect for housing condition ($F [3, 56] = 20.262$, $p < 0.001$), but no main effect for drug. The analysis also revealed a time by housing interaction ($F [22.176, 413.953] = 5.414$, $p < 0.001$). *Post-hoc* analyses revealed that the non-enriched, physically-enriched, and socially-enriched conditions all had greater amounts of activity than the super-enriched condition.

Exercise (see Figure 17). A two-way ANOVA revealed a main effect for housing ($F [3, 56] = 14.518$, $p < 0.001$) and a main effect for drug ($F [1, 56] = 7.687$, $p < 0.01$), but no housing by drug interaction. *Post hoc* analyses for the housing effect revealed that both non-enriched and physically-enriched animals engaged in greater amounts of exercise than super-enriched animals. Physically-enriched animals also engage in greater amounts of exercise than

socially-enriched animals. *Post hoc* analyses for the drug effect revealed that animals given nicotine engaged in greater amounts of exercise than animals given saline.

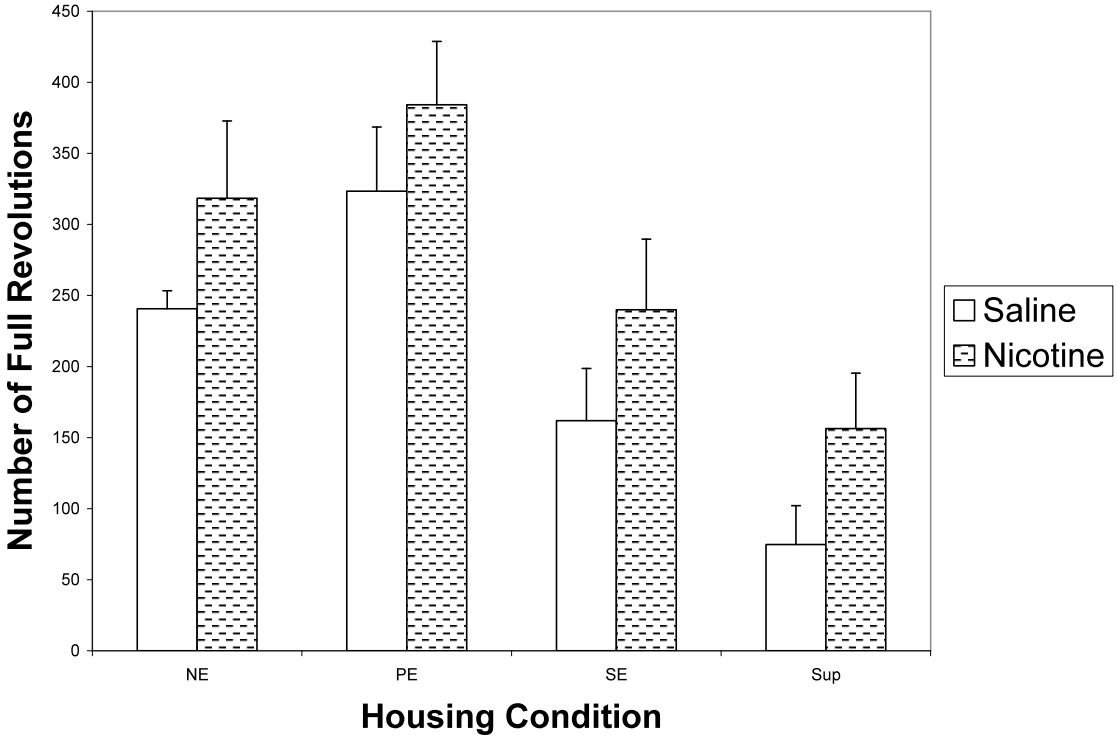

Figure 17. Mean voluntary exercise (± SEM) of male, Sprague Dawley rats in four different housing conditions and two different drug conditions (NE = non-enriched, PE = physically-enriched, SE = socially-enriched, Sup = super-enriched)

The assumption of sphericity was violated in the following analysis and the Greenhouse-Geisser correction was used to correct for this violation. Within-session repeated-measures ANOVA revealed a main effect for housing ($F [3, 56] = 14.573$, $p < 0.001$), a main effect for drug ($F [1, 56] = 7.638$, $p < 0.01$), and a

main effect for time ($F [1.239, 69.370] = 311.334$, $p < 0.001$). The within-session repeated-measures ANOVA also revealed two interactions: a time by housing interaction ($F [3.716, 69.370] = 13.227$, $p < 0.001$) and a time by drug interaction ($F [1.239, 39.370] = 6.854$, $p < 0.01$). *Post hoc* analyses revealed that both non-enriched and physically-enriched animals engaged in greater amounts of voluntary exercise than super-enriched animals. Physically-enriched animals also engaged in greater amounts of voluntary exercise than socially-enriched animals. Animals given nicotine engaged in greater amounts of voluntary exercise than animals given saline.

A two-way ANOVA for latency to start revealed no significant differences between groups. A repeated-measures ANOVA for number of full revolutions during the first minute of activity revealed no statistically significant differences between housing or drug conditions.

Discussion for Experiment IIb

The purpose of this experiment was to examine the effects of environmental enrichment's effects on nicotine cessation's effects on body weight, food consumption, and activity. Previous experiments have examined the effects of environmental enrichment on nicotine cessation. The previous study, however, used a different form of nicotine (nicotine bitartrate whereas this study used nicotine dihydrochloride), a different dose of nicotine (3.16 mg/kg/day whereas this study used 9 mg/kg/day), for a different length of time (7 days), and examined different dependent variables (focus was on nicotine cessation). The present experiment examined the dependent variables of body weight, food

consumption, open field activity, and voluntary exercise to determine the effects of various housing conditions on nicotine cessation.

Environmental enrichment's effects on body weight that were evident in Experiments I and IIa disappeared during Experiment IIb. Nicotine cessation decreased body weight gain. Neither environmental enrichment nor nicotine cessation affected food consumption.

Environmental enrichment decreased open field activity with super-enrichment having the greatest effects. Nicotine cessation *per se* did not affect open field activity, but environmental enrichment and nicotine cessation together affected open field activity. Nicotine cessation decreased open field vertical activity in the non-enriched group, but increased open field vertical activity in the super-enriched group.

Environmental enrichment had differential effects on exercise, depending on type and amount of enrichment. Physical enrichment increased exercise, whereas combined physical and social enrichment led to decreased voluntary exercise. Social enrichment alone did not seem to influence voluntary exercise, because it did not differ from non-enrichment in terms of amount of voluntary exercise. Nicotine cessation increased exercise, but there was no apparent interaction between housing and drug because nicotine cessation similarly increased activity across all environmental conditions

Environmental enrichment continued to have effects on activity, but no longer appeared to affect body weight or food consumption. Nicotine's effects persisted for body weight and voluntary exercise throughout the nicotine

cessation period. So, the independent variables (environmental enrichment and nicotine cessation) appeared to act independently of each other. With regard to body weight, the influence of environmental enrichment disappeared during nicotine cessation.

General Discussion

Several overall conclusions can be made from these experiments. Environmental enrichment effects on body weight cannot be fully accounted for by changes in food consumption and activity. Enrichment, specifically super-enrichment, resulted in attenuated weight gain. Super-enrichment, however, had minimal effects on food consumption and greatly *decreased* activity, including home cage activity, open field activity, and exercise. The attenuated weight gain therefore cannot be explained by observed differences in food consumption and activity. Based on the food consumption and activity findings alone, one would expect the super-enriched animals to display increased weight gain over time instead of attenuated weight gain.

Another conclusion that can be reached from these experiments is that voluntary exercise provides a valuable measure of activity that taps into an aspect of activity that differs from both home cage and open field activity. The two most interesting findings from voluntary exercise were that physical enrichment greatly increased and super-enrichment greatly decreased voluntary exercise. The relationship between enrichment and amount of voluntary exercise (PE>NE=SE>Sup) is different from all other measures and was unexpected. The effects of super-enrichment on voluntary exercise were striking because of the degree of effect. These findings, in comparison with the home cage and open field activity findings, indicate that different forms of enrichment affect different types of activity (including general movement and voluntary exercise activitity). No overall conclusion can be made on the effects of enrichment on activity.

However, it is clear that one type of activity (e.g., home cage movement) do not generalize to other forms of activity (e.g., voluntary exercise in a novel environment).

Environmental enrichment appears to alter some of chronic nicotine's effects on food consumption and open field activity, but not body weight or voluntary exercise. Environmental enrichment appeared to exacerbate nicotine's effects on food consumption (further decreasing food consumption) and appeared to exacerbate nicotine's effects on vertical activity in the open field chamber (further decreasing vertical activity). These differences in environmental enrichments varying effects on nicotine's actions indicate that they may work through the same mechanisms for some of these variables (i.e., food consumption and vertical activity) but not for all (i.e., body weight and voluntary exercise).

The last overall conclusion concerns enrichment itself. Previously, it seemed that a positive linear relationship existed between enrichment and performance on a variety of tasks - specifically, the greater the enrichment, the greater the performance. Instead, it appears that the relationship may be an inverse U-shaped curve, similar to the Yerkes-Dodson principle. Too much enrichment, therefore, may be detrimental to performance. This possibility deserves additional research attention because it may suggest how to optimize various health-related behaviors. This relationship also may differ across outcomes. For example, it appears that although super-enrichment has beneficial effects on body weight and learning (inferred from faster habituation in

the open field chamber), it does not increase activity. It is unclear as to whether these effects on activity are detrimental to health. Varying amounts of enrichment may be beneficial depending on the outcome examined.

Limitations

This project has several limitations. Methodologically, the method of nicotine administration is not an exact substitute for cigarette smoking. Although the chronic flow of nicotine better approximates the levels of nicotine found in smokers than daily acute injections of nicotine, it does not capture the fluctuations in smokers. The home cage activity measure was limited to approximately five minutes of observation every other day, which may not have been enough to capture the true level of home cage activity engaged in by the rats. The voluntary exercise measure also was limited in that the socially housed rats were separated from their cage mates during the measure, which may have accounted for some of the differences found between those rats who were housed individually and those who were housed socially. The decision to measure voluntary exercise, as opposed to forced exercise, is another limitation as it does not capture the full impact of enriched housing and nicotine on exercise. Voluntary exercise does not capture the animals' ability to exercise as forced exercise might. In fact, animals in this experiment engaged in voluntary exercise primarily during the first five minutes of the measurement period. The schedule by which the dependent variables were measures is another potential limitation. Some dependent variables were measured more often than others, which may have affected the findings. Furthermore, the division of the super-

enriched rats into two cages may also have affected the findings. Another limitation is that this project included only male, Sprague Dawley rats.

Clinical Implications

This project supports the notion that the environment can powerfully influence health risks and behaviors. Although the focus of health risk behaviors often rests on the individual, the environment has a large influence on engagement in health risk behaviors as well. If the present findings hold true for humans, then attempts to change health risk behaviors cannot focus solely on the individual, they must also include the environment. Specifically, moderate amounts of physical enrichment in the environment, such as exercise equipment, may lead to increased voluntary exercise in males. Large amounts of physical and social enrichment in the environment may lead to decreased body weight in males, but may have negative implications for physical activity. Because environmental influences contributed to the rise of chronic health problems in the United States, they must also be targeted to curb or reverse this trend.

Future Directions

Future directions primarily include addressing some of the limitations of this study. Using a 24 hour monitoring system to capture home cage activity would avoid the limitations encountered in this project and provide a more complete view of the effects of environmental enrichment and nicotine on home cage activity. Placing activity wheels in the home cage may remove the limitations of separating socially housed animals to measure exercise, but may introduce other complications. Measuring other forms of exercise, or including

forced exercise or motivated exercise (i.e., rewarding an animal after exercising), may provide greater insight into how enrichment and nicotine may affect not only exercise behavior, but perhaps exercise motivation as well. Another future direction is to include females and other strains of rats, providing insight into how hormones and genetic differences may impact the findings of this project.

Extensions of this study are limited largely by feasibility and methodological difficulties. For example, a human version of this study would not entertain the same level of experimental control and would likely be largely an observational study, although it would also have greater face validity and take into account the psychosocial aspects of the human experience. The psychosocial aspects would, however, greatly increase the number of variables to be accounted for and would likely limit the interpretations that might be made. The difficulties in interpretation and experimental control would outweigh any benefits derived from completing a similar study in humans.

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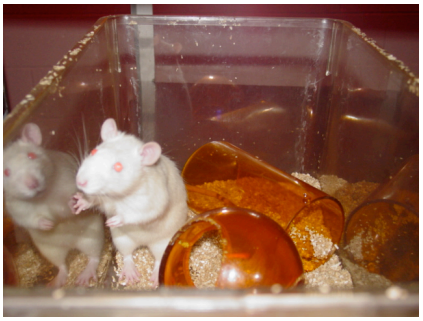
Appendix A: Housing Pictures

Non-enriched housing



Physically-enriched housing

Socially-enriched housing

Super-enriched housing

Appendix B: Home Cage Activity Rating Form

Home Cage Activity (1 Minute Observations)

Condition: _____ Rater Initials: _____

Circle a number between 1 and 7.

Number of Animals Moving

1	2	3	4	5	6	7
None	1-3	4-6	7-9	10-12	13-15	16

Amount of Activity for Majority of Group Members

1	2	3	4	5	6	7
None	Almost No Activity	Low Activity	Some Activity	Moderate Activity	Intermittent High Activity	Continuous High Activity

Level of Activity

1	2	3	4	5	6	7
None	Almost No Effort	Low Effort	Some Effort	Moderate Effort	Intermittent High Effort	Continuous High Effort

Indicate the type of activity and the number of animals engaged in each type of activity:

w/ Physical Object	Social Interaction	Combined P & S	Alone
_____	_____	_____	_____

Description/Comments:_____
_____
_____
_____
_____

Appendix C : Experiment I Tables

Table 1 - Body Weight Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	1.110 (3, 60)	0.352
	Time	2504.784 (4, 240)	< 0.001
	Housing x Time	2.148 (12, 240)	0.049

Table 2 - Food Consumption Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	1.139 (3, 58)	0.341
	Time	2534.79 (1.967, 114.101)	< 0.001
	Housing x Time	7.353 (5.902, 114.101)	< 0.001

Table 3 - Home Cage Activity Number of Animals Moving Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	8.365 (3, 18)	0.001
	Time	7.420 (4, 72)	< 0.001
	Housing x Time	1.427 (12, 72)	0.174

Table 4 - Home Cage Activity Amount of Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	6.971 (3, 19)	0.002
	Time	3.432 (2.074, 39.401)	0.041
	Housing x Time	0.694 (6.221, 39.401)	0.661

Table 5 - Home Cage Activity Effort of Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	5.840 (3, 19)	0.005
	Time	3.849 (2.322, 44.116)	0.023
	Housing x Time	0.683 (6.966, 44.116)	0.685

Table 6 - Open Field Activity Horizontal Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	28.333 (3, 60)	< 0.001
	Time	2.289 (1, 60)	0.136
	Housing x Time	0.098 (3, 60)	0.961

Table 7 - Open Field Activity Vertical Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	2.509 (3, 60)	0.067
	Time	0.004 (1, 60)	0.948
	Housing x Time	0.902 (3, 60)	0.446

Table 8 - Open Field Activity Center Time Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	3.190 (3, 60)	0.030
	Time	12.026 (1, 60)	0.001
	Housing x Time	1.999 (3, 60)	0.124

Table 9 - Open Field Week 1 Within Session Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	20.982 (3, 60)	< 0.001
	Time	198.759 (4.171, 250.233)	< 0.001
	Housing x Time	9.733 (12.512, 250.233)	< 0.001

Table 10 - Open Field Week 3 Within Session Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	16.293 (3, 60)	< 0.001
	Time	202.089 (5.316, 318.939)	< 0.001
	Housing x Time	5.878 (15.947, 318.939)	< 0.001

Table 11 - Exercise Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	10.118 (3, 60)	< 0.001
	Time	2.899 (1, 60)	0.094
	Housing x Time	2.491 (3, 60)	0.069

Table 12 - Exercise Week 2 Within Session Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	8.616 (3, 60)	< 0.001
	Time	143.932 (1.185, 71.083)	< 0.001
	Housing x Time	8.183 (3.554, 71.083)	< 0.001

Table 13 - Exercise Week 4 Within Session Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	9.175 (3, 60)	< 0.001
	Time	138.587 (1.211, 72.633)	< 0.001
	Housing x Time	8.434 (3.632, 72.633)	< 0.001

Table 14 - Exercise Latency to Start Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	0.871 (3, 60)	0.461
	Time	6.870 (1, 60)	0.011
	Housing x Time	1.125 (3, 60)	0.346

Table 15 - Exercise Number of Full Revolutions During Start Minute Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	8.634 (3, 60)	< 0.001
	Time	145.377 (1, 60)	< 0.001
	Housing x Time	8.578 (3, 60)	< 0.001

Appendix D: Experiment IIa Drug Phase Tables

Table 1 - Body Weight Repeated Measures ANCOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	3.227 (3, 55)	0.029
	Drug	129.669 (1, 55)	< 0.001
	Time	0.206 (1.177, 64.722)	0.691
	Covariate	1057.907 (1, 55)	< 0.001
	Housing x Drug	0.584 (3, 55)	0.628
	Housing x Time	1.238 (3.530, 64.722)	0.304
	Drug x Time	16.701 (1.177, 64.722)	< 0.001
	Covariate x Time	4.332 (1.177, 64.722)	0.035
	Time x Housing x Drug	1.078 (3.530, 64.722)	0.371

Table 2 - Food Consumption Repeated Measures ANCOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	0.936 (3, 54)	0.430
	Drug	73.150 (1, 54)	< 0.001
	Time	0.707 (1.744, 94.187)	0.477
	Covariate	23.549 (1, 54)	<0.001
	Housing x Drug	2.732 (3, 54)	0.053
	Housing x Time	2.529 (5.233, 94.187)	0.032
	Drug x Time	37.822 (1.744, 94.187)	< 0.001
	Covariate x Time	0.358 (1.744, 94.187)	0.671
	Time x Housing x Drug	2.371 (5.233, 94.187)	0.043

Table 3 - Open Field Horizontal Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	32.142 (3, 56)	< 0.001
	Drug	2.987 (1, 56)	0.089
	Time	1.169 (1, 56)	0.284
	Housing x Drug	1.888 (3, 56)	0.142
	Time x Housing	0.446 (3, 56)	0.721
	Time x Drug	0.059 (1, 56)	0.810
	Time x Housing x Drug	0.372 (3, 56)	0.773

Table 4 - Open Field Vertical Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	17.837 (3, 56)	< 0.001
	Drug	8.738 (1, 56)	0.05
	Time	12.991 (1, 56)	0.001
	Housing x Drug	3.429 (3, 56)	0.023
	Time x Housing	0.767 (3, 56)	0.517
	Time x Drug	0.039(1, 56)	0.884
	Time x Housing x Drug	0.236 (3, 56)	0.871

Table 5 - Open Field Center Time Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	3.329 (3, 56)	0.026
	Drug	0.777 (1, 56)	0.382
	Time	3,159 (1, 56)	0.081
	Housing x Drug	1.390 (3, 56)	0.255
	Time x Housing	5.227 (3, 56)	0.003
	Time x Drug	0.143 (1, 56)	0.707
	Time x Housing x Drug	0.289 (3, 56)	0.833

Table 6 - Open Field Week 5 Within Session Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	16.317 (3, 55)	< 0.001
	Drug	1.003 (1, 55)	0.321
	Time	171.765 (5.839, 321.152)	< 0.001
	Housing x Drug	0.918 (3, 55)	0.438
	Time x Housing	3.632 (17.517, 321.152)	< 0.001
	Time x Drug	1.813 (5.839, 321.152)	0.098
	Time x Housing x Drug	0.990 (17.517, 321.152)	0.470

Table 7 - Open Field Week 7 Within Session Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	28.782 (3, 56)	< 0.001
	Drug	3.436 (1, 56)	0.069
	Time	193.369 (7.020, 393.099)	< 0.001
	Housing x Drug	2.023 (3, 56)	0.121
	Time x Housing	2.369 (21.059, 393.099)	< 0.001
	Time x Drug	1.691 (7.020, 393.099)	0.109
	Time x Housing x Drug	1.548 (21.059, 393.099)	0.059

Table 8 - Exercise Two-Way ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	2.712 (3, 56)	0.053
	Drug	7.249 (1, 56)	0.009
	Housing x Drug	0.090 (3, 56)	0.965

Table 9 - Exercise Week 6 Within Session Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	2.708 (3, 56)	0.054
	Drug	7.260 (1, 56)	0.009
	Time	156.970 (1.160, 64.978)	< 0.001
	Housing x Drug	0.088 (3, 56)	0.966
	Housing x Time	2.851 (3.481, 64.978)	0.037
	Drug x Time	6.543 (1.160, 64.978)	0.010
	Housing x Drug x Time	0.132 (3.481, 64.978)	0.957

Table 10 - Exercise Latency to Start Two-Way ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	2.710 (3, 56)	0.054
	Drug	0.452 (1, 56)	0.504
	Housing x Drug	4.968 (3, 56)	0.004

Table 11 - Exercise Number of Full Revolutions During Start Minute Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	1.042 (3, 56)	0.381
	Drug	2.645 (1, 56)	0.110
	Housing x Drug	1.087 (3, 56)	0.362

Appendix E: Experiment IIb Post-Drug Phase Tables

Table 1 - Body Weight Repeated Measures ANCOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	0.156 (3, 55)	0.925
	Drug	19.534 (1, 55)	< 0.001
	Time	1.651 (1.649, 90.686)	0.201
	Covariate	791.937 (1, 55)	<0.001
	Housing x Drug	4.818 (3, 55)	0.005
	Housing x Time	2.146 (4.947, 90.686)	0.054
	Drug x Time	14.817 (1.649, 90.686)	< 0.001
	Covariate x Time	12.946 (1.649, 90.686)	< 0.001
	Time x Housing x Drug	0.783 (4.947, 90.686)	0.563

Table 2 - Food Consumption Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	0.551 (3, 53)	0.649
	Drug	2.004 (1, 53)	0.163
	Time	1.236 (1.627, 86.229)	0.283
	Covariate	10.040 (1, 53)	0.003
	Housing x Drug	1.976 (3, 53)	0.129
	Housing x Time	1.446 (4.881, 86.229)	0.217
	Drug x Time	0.709 (1.627, 86.229)	0.467
	Covariate x Time	2.086 (1.627, 86.229)	0.139
	Time x Housing x Drug	1.955 (4.881, 86.229)	0.095

Table 3 - Open Field Horizontal Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	24.877 (3, 56)	< 0.001
	Drug	0.665 (1, 56)	0.418
	Time	0.241 (1, 56)	0.626
	Housing x Drug	1.697 (3, 56)	0.178
	Time x Housing	0.034 (3, 56)	0.991
	Time x Drug	0.434 (1, 56)	0.513
	Time x Housing x Drug	0.320 (3, 56)	0.811

Table 4 - Open Field Vertical Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	9.308 (3, 56)	< 0.001
	Drug	3.295 (1, 56)	0.075
	Time	3.805 (1, 56)	0.056
	Housing x Drug	5.727 (3, 56)	0.002
	Time x Housing	6.193 (3, 56)	0.001
	Time x Drug	0.001 (1, 56)	0.974
	Time x Housing x Drug	1.200 (3, 56)	0.318

Table 5 - Open Field Center Time Activity Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	3.023 (3, 56)	0.037
	Drug	0.348 (1, 56)	0.557
	Time	0.781 (1, 56)	0.381
	Housing x Drug	2.696 (3, 56)	0.055
	Time x Housing	0.222 (3, 56)	0.861
	Time x Drug	0.064 (1, 56)	0.801
	Time x Housing x Drug	1.021 (3, 56)	0.390

Table 6 - Open Field Week 8 Within Session Repeated Measures ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	18.075 (3, 56)	< 0.001
	Drug	0.151 (1, 56)	0.699
	Time	267.726 (7.157, 400.817)	< 0.001
	Housing x Drug	1.891 (3, 56)	0.142
	Time x Housing	6.016 (21.472, 400.817)	0.001
	Time x Drug	1.800 (7.157, 400.817)	0.084
	Time x Housing x Drug	1.025 (21.472, 400.817)	0.431

Table 7 - Open Field Week 10 Within Session Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	20.262 (3, 56)	< 0.001
	Drug	1.116 (1, 56)	0.295
	Time	217.414 (7.392, 413.953)	< 0.001
	Housing x Drug	0.816 (3, 56)	0.490
	Time x Housing	5.414 (22.176, 413.953)	< 0.001
	Time x Drug	1.875 (7.392, 413.953)	0.068
	Time x Housing x Drug	0.688 (22.176, 413.953)	0.854

Table 8 - Exercise Two-Way ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	14.518 (3, 56)	< 0.001
	Drug	7.687 (1, 56)	0.008
	Housing x Drug	0.030 (3, 56)	0.993

Table 9 - Exercise Week 9 Within Session Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	14.573 (3, 56)	< 0.001
	Drug	7.638 (1, 56)	0.008
	Time	311.334 (1.239, 69.370)	< 0.001
	Housing x Drug	0.029 (3, 56)	0.993
	Housing x Time	13.227 (3.716, 69.370)	< 0.001
	Drug x Time	6.854 (1.239, 69.370)	0.007
	Housing x Drug x Time	0.193 (3.716, 69.370)	0.932

Table 10 - Exercise Latency to Start Two-Way ANOVA			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	1.222 (3, 56)	0.310
	Drug	0.333 (1, 56)	0.566
	Housing x Drug	0.333 (3, 56)	0.801

Table 11 - Exercise Number of Full Revolutions During Start Minute Repeated Measures ANOVAs			
Experimental Group	**Effect**	**F value (df)**	**P value**
All Animals	Housing	1.699 (3, 56)	0.178
	Drug	1.517 (1, 56)	0.223
	Housing x Drug	1.983 (3, 56)	0.127